Developmental morphological diversity in caecilian amphibians: systematic and evolutionary implications

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Cover illustration: Embryo of *Ichthyophis* cf. *kohtaoensis* cleared and stained for bone (red) and cartilage (blue).
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DEVELOPMENTAL MORPHOLOGICAL DIVERSITY IN CAECILIAN AMPHIBIANS: SYSTEMATIC AND EVOLUTIONARY IMPLICATIONS

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SUMMARY

Caecilians, or Gymnophiona, constitute one of the three extant orders of the Recent Amphibia and comprise about 170 named species in six families. They are the least known, major living tetrapod clade, which is probably mainly due to their largely subterranean habits and confinement to parts of the wet and seasonal tropics of South and Middle America, Africa and Asia. Because of their unusual skull morphology, as compared to frogs and salamanders, caecilians received considerable interest from early morphologists. With few exceptions, however, most studies on caecilian morphology have been restricted to investigations on adult material and were usually carried out on small samples. This led to uncertainties about bone homologies, because the heavily ossified, burrowing-adapted caecilian skulls are highly modified compared to the skulls of other amphibians, which in turn influenced hypotheses on the phylogenetic position of caecilians. I studied the development of the skull to: a) investigate what bones the caecilian skull is formed of and what their homologues are compared to other amphibians, b) survey and document the developmental diversity of caecilians and the evolution of cranial ontogeny and c) investigate the influences of different life-history strategies on skull development.

For a detailed investigation of caecilian skull morphology, I studied the development of the skull in *Hypogeophis rostratus*, a more derived caeciliid caecilian with direct development (Chapter 2). My results are in conflict with earlier studies (e.g. Marcus et al., 1935) that reported a much higher number of individual bones and their later fusion. In *H. rostratus* (and all other investigated taxa) no evidence is found for several of the reported ossifications, including supra-, infra- and basioccipital, epiotic, pleurosphenoid, preethmoid, posterior vomer, prepalatine, quadratojugal, postparietal, second coronoid, supraangular and complementary. Their previous description by Marcus et al. (1935) has been hugely influential in subsequent studies of caecilian skull morphology and amphibian evolution. Here it is argued that most of Marcus et al.'s reports of non-existent ossifications are based on false phylogenetic preconception,

misinterpretation of the observed morphology and technical error. No evidence is found that would argue for a close relationship with certain Paleozoic forms, such as Microsauria.

The plesiomorphic life history in caecilians, as in other amphibians, is oviparity with a free-living larva that undergoes a metamorphosis to transform into the adult-like morphology. The extent of metamorphic change that occurs during development, however, is largely unknown and very few descriptions of larval morphology exist of rhinatrematid and ichthyophiid larvae and virtually no information is available on the osteology and myology of those of uraeotyphlids and caeciliids. In chapter 3, the larval morphology and metamorphic changes in skull morphology and cranial musculature of rhinatrematid, ichthyophiid, uraeotyphlid and caeciliid (*Sylvacaecilia, Grandisonia, Praslinia*) caecilians are investigated, representing all genera for which free-living larvae are known. Based on both osteology and myology, it is argued that caecilians are derived from gymno- or zygokrotaphic ancestors and that stegokrotaphy, which is characteristic of the adult skull of most caecilians, evolved within Recent caecilians.

To investigate the influence of different life-histories on early skull development, the postembryonic development of the skull in the direct-developing caeciliids *Boulengerula taitanus* was studied and compared with that of the direct developing *Gegeneophis ramaswamii* and the more basal branching *Ichthyophis* cf. *kohtaoensis*, a form with free-living aquatic larvae. While hatchling *G. ramaswamii* have well-ossified skulls and resemble a miniature adult, *B. taitanus* has very immature hatchlings that have a similar degree of skull ossification to that of larval *Ichthyophis* sp. This is correlated with an extended period of post-hatching parental care in *B. taitanus*, during which juveniles triple in size and feed on the specially modified stratum corneum of their mothers' skin (Chapter 5). Direct development in caecilians is further characterized by ontogenetic repatterning and heterochronic shifts in certain developmental events compared to the more plesiomorphic ontogeny exemplified by *I.* cf. *kohtaoensis*.

As representative of a viviparous species, the ontogeny of the scolecomorphid *Scolecomorphus kirkii* was studied (Chapter 6). Foetuses and juveniles of *S. kirkii* show a highly unusual skull morphology that is characterized by a massively developed endocranium and a unique configuration of the dental arcades. These and other characteristics of young *Scolecomorphus* are indicative of a highly specialized life-history stage.

Chapter 7 provides a summary and synthesis of the thesis. The emerging picture of available published information, together with observations presented here, suggest a previously unsuspected degree of developmental diversity among caecilians, and the available phylogenetic data imply frequent independent evolution of derived reproductive traits such as viviparity and possibly also direct development. Based on developmental data it is further argued that caecilians are lissamphibians with temnospondyl affinities rather than closely related to lepospondyl microsaurs.

SAMENVATTING

De wormsalamanders, of Caecilia, vormen één van de drie ordes van de klasse Amfibieën en omvatten ongeveer 170 soorten die onderverdeeld zijn in zes families. Het is de minst bekende, nog levende tetrapoden groep, wat waarschijnlijk te wijten is aan hun grotendeels ondergrondse levenswijze in Zuid en Midden Amerika, Afrika en Azië. Omdat hun schedel morfologie afwijkt van die van kikkers en salamanders, trokken wormsalamanders reeds vroeg de aandacht van morfologen. De meeste morfologische studies tot nu hebben zich gericht op volwassen exemplaren van wormsalamanders. Hierdoor zijn er onzekerheden wat betreft de homologie der beenstructuren omdat de zwaar verbeende schedels, aangepast aan het graven, enorm veranderd zijn in vergelijking met de schedels van andere amfibieën. Dit beïnvloedde de hypotheses omtrent de fylogenetische positie van wormsalamanders. Ik bestudeerde de ontwikkeling van de schedel om a) na te gaan uit welke beenderen de schedel van de wormsalamander gevormd is en welke hun homologien zijn in vergelijking met andere amfibieën, en b) de diversiteit in ontwikkeling van wormsalamanders te bestuderen en documenteren, en de invloed van verscheidene levensgeschiedeniskenmerken op vroege ontogenese te onderzoeken.

Voor een gedetailleerde studie van de schedel morfologie in wormsalamanders bestudeerde ik in hoofdstuk twee de schedelontwikkeling in *Hypogeophis rostratus*, een verder gevorderde soort wormsalamander met directe ontwikkeling. Mijn resultaten zijn in tegenspraak met vroegere studies (zoals Marcus et al., 1935) die een veel groter aantal individuele beenderen en een latere vergroeiing beschreven. In *H. rostratus* (en alle andere onderzochte taxa) werd er geen bewijs gevonden voor de vermelde verbeningen, zoals de supra-, infra- en basioccipitale, epioticum, pleurosphenoideum, praeethmoideum, posterior vomer, praepalatinum, quadratojugale, postparietale, tweede coronoide, supraangulaire en complementare. De vorige beschrijving door Marcus et al. (1935) had een enorme invloed op de daaropvolgende studies omtrent schedelmorfologie van

wormsalamanders en de evolutie van amfibieën. Hier wordt er geargumenteerd dat de meeste beschrijvingen van Marcus en collegae over niet-bestaande verbeningen gebaseerd zijn op foute fylogenetische veronderstellingen, misinterpretatie van de geobserveerde morfologie en technische fouten. Er is geen bewijs gevonden voor een nauwe verwantschap met bepaalde paleozoische vormen, zoals de Microsauria.

De plesiomorfe levensgeschiedenisvorm van wormsalamanders vergelijkbaar met die van andere amfibieën, namelijk eierleggend met een vrijlevende larve die een metamorfose ondergaat naar een vorm gelijkend op het volwassen stadium. De mate van verandering in die metamorfose is echter grotendeels onbekend, want en er bestaan zeer weinig beschrijvingen van larvale morfologie in rhinatrematidae en ichthyophiidae larven, en er is bijna helemaal geen informatie voorhanden over de osteologie en myologie van de soorten behorende tot *Uraeotyphlidae* en *Caeciliidae*. Hoofdstuk 3 beschrijft de larvale morfologie en veranderingen die optreden gedurende de metamorfose in schedel morfologie en craniale musculatuur van wormsalamanders behorend tot de *Rhinatrematidae*, *Ichthyophiidae*, *Uraeotyphlidae* en de *Caeciliidae* (*Sylvacaecilia, Grandisonia, Praslinia*), alle genera waarbij vrijlevende larven beschreven zijn. Gebaseerd op zowel osteologie als myologie, wordt verondersteld dat wormsalamanders afstammen van gymno- of zygokrotafische voorouders en dat stegokrotafie, kenmerkend voor de volwassen schedel van de meeste wormsalamanders, geëvolueerd is binnen de recente amfibieën

Om de invloed van verscheidene levensgeschiedeniskenmerken op vroege schedelontwikkeling te onderzoeken, werd de postembryonale schedelontwikkeling in de wormsalamander met directe ontwikkeling, *Boulengerula taitanus*, bestudeerd en vergeleken met deze in *Gegeneophis ramaswamii* en de meer basaal aftakkende *Ichthyophis* cf. *kohtaoensis*, een soort met een vrijlevende aquatische larve. Waar jongen van *G. ramaswamii* een goed verbeende schedel hebben, gelijkend op een volwassen stadium, heeft *B. taitanus* zeer onvolgroeide jongen met een gelijkaardig patroon van schedel verbening als de jongen van *Ichthyophis* sp. Dit is gecorreleerd met een verlengde periode van

ouderzorg bij *B. taitanus*, gedurende welke juvenielen verdriedubbelen in grootte en zich voeden met de speciaal aangepaste hoornlaag (*stratum corneum*) van hun moeders (Hoofdstuk 5). Directe ontwikkeling in wormsalamanders wordt voorts gekenmerkt door ontogenische 'repatterning' en heterochronische verschuivingen in bepaalde ontwikkelingsgebeurtenissen in vergelijking met de meer plesiomorfe ontogenie bij *I.* cf. *kohtaoensis*.

Als voorbeeld van een levendbarende soort werd de ontogenie van de scolecomorphide *Scolecomorphus kirkii* bestudeerd (Hoofdstuk 6). Foetussen en juvenielen van *S. kirkii* vertonen een hoogst ongewone schedelmorfologie die gekarakteriseerd wordt door een massief ontwikkeld endocranium en een unieke configuratie van de dentale arcaden. Deze en andere kenmerken van jonge *Scolecomorphus* wijzen op een zeer gespecialiseerde ontwikkeling.

Hoofdstuk 7 geeft een samenvatting en synthese van het proefschrift. De informatie uit de literatuur en de observaties die hier beschreven zijn, suggereren een ongekende diversiteit in ontwikkelingsvormen in wormsalamanders, en de fylogenetische data voorhanden wijzen op frequente onafhankelijke evoluties van afgeleide voortplantingskenmerken zoals levendbarendheid en mogelijk ook directe ontwikkeling. De ontwikkelingsdata in acht nemend, kan er verder geargumenteerd worden dat wormsalamanders beschouwd kunnen worden als Lissamphibia met temnospondyle affiniteiten in plaats van een nauwe verwantschap met lepospondyle Microsauria.

CHAPTER 1 – INTRODUCTION

Caecilian amphibians (Gymnophiona) – an introduction

Caecilian or Gymnophiona, together with frogs (Anura) and salamanders (Caudata), constitute the three living orders of the Amphibia. Caecilians are often thought of as the least known major group of tetrapods, and certainly of the three living orders of amphibians. They are elongated snake-like amphibians completely lacking limbs and girdles and they have a primarily terrestrial, surface-cryptic or burrowing lifestyle as adults, except for the Typhlonectidae, a South America group that are secondarily aquatic or semiaquatic (Taylor, 1968; Wilkinson and Nussbaum, 1999). The majority of the approximately 170 recognized species inhabit the wet tropics of Central and South America, Africa and Asia, with some species also reaching the subtropics of South America and Asia (Wilkinson and Nussbaum, 2006). The current distribution is commonly accepted to reflect an Gondwanan origin of the group (Duellman and Trueb, 1986; Hedges et al., 1993; Wilkinson et al., 2002; San Mauro et al., 200).

Compared to frogs and salamanders, very little information is available on many aspects of caecilian biology (see Himstedt, 1996 for most comprehensive recent review). Their secretive, mostly burrowing lifestyle and tropical distribution are very likely the main reason that caecilians are rarely encountered in the field, unless special sampling effort is made (e.g. Malonza and Müller, 2004; Measey, 2004; Gower and Wilkinson, 2005), and thus likely responsible for their poor representation in museum collections. Many morphological specialisations of caecilians are also attributable to their burrowing lifestyle, including their compact and heavily ossified skull that is unlike that of salamanders and especially frogs (Taylor, 1969a; Trueb, 1993). Caecilians possess a unique sensory organ, the tentacle, which is derived from structures of the eye and associated musculature and glands (Sarasin and Sarasin, 1887-1890; Billo and Wake, 1987) and which serves as chemo-mechanosensory organ that is probably used by the animal to detect surface-borne scent molecules and also to orient itself within its burrow (Himstedt and Simon, 1995). Six families of

caecilians are currently recognized (Wilkinson and Nussbaum, 2006; but see Frost et al. 2006). Although caecilians are relatively uniform in their external appearance (Himstedt, 1996), recent research has uncovered a remarkable degree of morphological (e.g. Nussbaum and Wilkinson, 1995; Wilkinson, 1992a; Gower and Wilkinson, 2002), ecological (e.g. Gower et al., 2004, Jones et al., 2006), and life history diversity (Wake, 1977; Loader et al., 2003; Kupfer et al., 2004, 2006;).

The fossil record of caecilians is poor and consists exclusively of isolated vertebrae of uncertain affinities from the Palaeocene of Brazil and Bolivia (Estes and Wake, 1972; Rage, 1986; Rage, 1991), the Upper Cretaceous of Sudan (Evans et al., 1996; Werner, 1994) and the Miocene of Colombia (Hecht and LaDuke, 1997). Further fossil vertebrae referred to *Dermophis* are known from the Quaternary of Mexico (Wake et al., 1999). Two putative stem-group caecilians exist. The older, *Eocaecilia micropodia* from the Lower Jurassic of Arizona, USA, is known from about 30 specimens of variable completeness. It possesses limbs, albeit reduced in size, and shows a modestly elongated trunk (Jenkins and Walsh, 1993). The second taxon, *Rubricacaecilia monbaroni* from the Lower Cretaceous of Morocco, is known from several isolated bone fragments, and it might also have limbs (Evans and Sigogneau-Russel, 2001). Recent time tree analyses suggest that the crown group had already started to diversify at the time the aforementioned stem group representatives existed (San Mauro et al., 2005; Roelants et al., 2007).

Phylogenetic relationships of caecilians

The phylogenetic relationships of caecilians with regard to other amphibians have been a matter of debate for well over a century. They were initially considered to be degenerate snakes, until Johannes Müller (1831a) discovered gill slits in a larva of an ichthyophiid and thus established that caecilians are amphibians. The exact relationships of caecilians to other amphibians, however, remained controversial. Some authorities considered caecilians to be closely related to certain Palaeozoic forms (e.g. Wiedersheim, 1879, Kingsley, 1902), whereas

others considered them to be closely related to, or even within, salamanders (Sarasin and Sarasin, 1887-1890). In 1901, Gadow proposed the Lissamphibia concept in its current usage: all three Recent orders of living amphibians form a monophyletic group that arose from a single lineage of Palaeozoic amphibians. This was in contrast to Haeckel (1866) who originally proposed Lissamphibia as the name for the group comprising frogs and salamanders, to the explicit exclusion of caecilians. The Lissamphibia concept (sensu Gadow, 1901), however, was largely ignored during the first half of the 20th century and many researchers propagated a closer relationship of caecilians with various Palaeozoic groups, than with frogs and salamanders. The most prominent of these were Harry Marcus and his students, who studied various aspects of caecilian anatomy and development, and considered caecilians to be living representatives of so-called stegocephalian amphibians, in particular aistopods (e.g. Eifertinger, 1933; Marcus, 1933; Marcus et al., 1935).

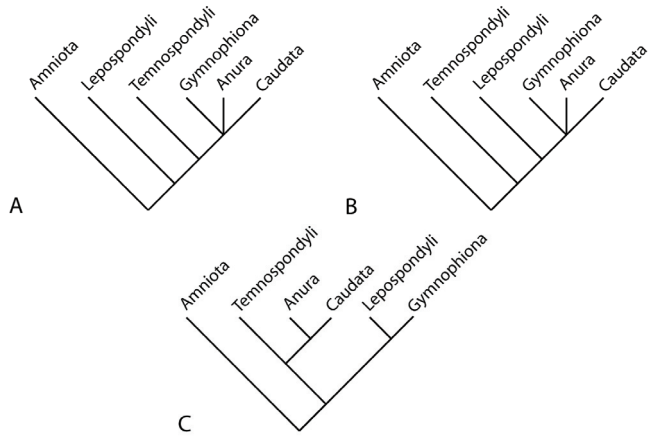

Fig. 1. Phylogenetic relationships of living amphibians: **A** Temnospondyls are ancestors of a monophyletic Lissamphibia, **B** Lepospondyli are ancestors of a monophyletic Lissamphibia, **C** Lissamphibians are diphyletic, frogs and salamanders related to temnospondyls, caecilians related to lepospondyls. See text for further

In 1963, Parsons and Williams revived the Lissamphibia as a monophyletic group comprising all Recent amphibians and provided a large number of characters in support of their monophyly. Most subsequent workers have accepted the Lissamphibia although different Palaeozoic groups have been

proposed as being ancestral to the Lissamphibia (see Schoch and Milner, 2004, for most recent comprehensive review). The majority of studies considered Lissamphibians to be derived from Temnospondyli (e.g. Parsons and Williams, 1963; Milner, 1988; Bolt, 1991; Trueb and Cloutier, 1991; Ruta et al., 2003; Schoch and Milner, 2004), whereas Laurin (1998) and Laurin and Reisz (1997) recently proposed an origin of the Lissamphibia from among the Lepospondyli. Other authors, however, considered Lissamphibia to be paraphyletic with regard to Palaeozoic amphibians and considered frogs, salamanders and caecilians to be derived from different Palaeozoic lineages. According to this so-called diphyly hypothesis, frogs and salamanders arose from temnospondyl dissorophoids and caecilians from lepospondyl microsaurs (Carroll, 2000; Carroll et al., 2004; see Fig. 1C), with which they share at least a superficially similar skull morphology (see Fig. 4). Nussbaum (1983) considered lepospondyl lysorophids to be the closest relatives of caecilians. Løvtrup (1985), however, considered caecilians to be more closely related to amniotes than to other caecilians, while Jarvik (1980) considered frogs on the one hand and salamanders and caecilians on the other to be independently derived from osteolepiform and, respectively, porolepiform sarcopterygians. Both hypotheses received no subsequent support and are not currently in fashion.

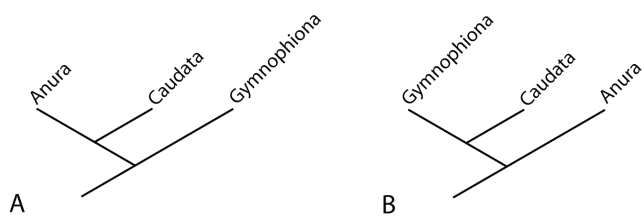

Fig. 2. A The Batrachia hypothesis: frogs and salamanders are sistergroups to the exclusion of caecilians. **B** The Procera hypothesis: caecilians and salamanders form a monophyletic group to the exclusion of frogs.

Two competing hypotheses currently exist regarding the relationships of the three lissamphibian groups – frogs, salamanders and caecilians – to each other. Most studies based on both morphological (e.g. Rage and Janvier, 1982; Milner, 1988; McGowan and Evans, 1995; Trueb and Cloutier, 1991) and molecular data (e.g. Venkatesh et al. 2001; Zardoya and Meyer 2001; San Mauro

et al 2005; Roelants et al 2007) support frogs as the sister group of salamanders, to the exclusion of caecilians (Fig. 1A). The clade comprising frogs and salamanders has been termed Batrachia (Milner, 1988). Earlier molecular studies (e.g. Hedges et al., 1990; Hedges and Maxson, 1993; Feller and Hedges, 1998), which used comparatively small datasets relative to more recent studies (e.g. San Mauro et al 2005; Roelants et al 2007), recovered caecilians as the sister group of salamanders, to the exclusion of frogs (Fig. 1B). This Procera hypothesis, named for the clade comprising caecilians and salamanders, also received some limited morphological support based on the skeletal anatomy of *Eocaecilia micropodia*, a putative stem group caecilian (Jenkins and Walsh, 1993). The question of the relationships among the three living orders and to Palaeozoic groups is further compounded by the poor fossil record of frogs, salamanders and especially caecilians. It is obvious that all three groups are very different in their general morphology and many aspects of their biology, and that each group seems to have acquired their specialized morphology at a very early point in their evolutionary history (Zardoya and Meyer, 2001; Schoch and Milner, 2004; Roelants et al., 2007), with hardly any plausible intermediates being currently known.

Caecilian intrarelationships

Although caecilians made an early appearance in the scientific literature (Seba, 1735), their alpha- and higher level diversity was long presumed to be relatively low. An important early student of caecilian systematics was Wilhelm C. H. Peters, who described several new species and genera and also provided the first hypothesis of caecilian intrarelationships (e.g. Peters, 1880). During the first half of the 20th century Emmett R. Dunn (e.g. 1942) made important contributions to the systematics and taxonomy of American caecilians while Arthur Loveridge (e.g. Loveridge, 1936) and especially H. W. Parker (e.g. 1936; 1958) advanced the understanding of African caecilians. The most important contribution to caecilian taxonomy was made by Edward H. Taylor, who not only revised and described many of the currently recognized species (e.g. Taylor 1960, 1968, 1969b), but also erected the families Ichthyophiidae and Typhlonectidae (Taylor,

1968), and Scolecomorphidae (Taylor, 1969c). Subsequently, Nussbaum (1977) recognized the Rhinatrematidae as a family distinct from ichthyophiid caecilians. In 1979, Nussbaum erected the family Uraeotyphlidae to accommodate the genus *Uraeotyphlus*, which had previously been placed in the Caeciliidae. Four of these families are relatively small and have more restricted distributions: the Rhinatrematidae (two genera, nine species) and Typhlonectidae (five genera, 13 species) occur in South America, the Uraeotyphlidae (one genus, five species) in India, while the Scolecomorphidae (two genera, six species) are confined to mountainous areas of East and West Africa (Wilkinson and Nussbaum, 2006). The second largest family, the Ichthyophiidae (two genera, 37 species) occur in South and South East Asia. By far the largest and most heterogeneous family is the Caeciliidae (21 genera, 100+ species), which occur in Central and South America, Africa, the Seychelles and India (Wilkinson and Nussbaum, 2006).

In contrast to frogs and salamanders, where various hypothesis of familial relationships have been proposed (e.g. Ford and Cannatella, 1993; Weisrock et al., 2005; Wiens et al., 2005; Frost et al., 2006), estimates of familial relationships in caecilians have been relatively stable. There is numerous morphological and molecular evidence that Rhinatrematidae are the sister group to all other living caecilians (e.g. Nussbaum, 1977; Wilkinson, 1992b; Wilkinson, 1996; Hedges et al., 1993; San Mauro et al. 2005; Roelants et al. 2007). Nussbaum (1979), and Duellman and Trueb (1986) and Hillis (1991), using family level taxa and a subset of characters from Nussbaum (1979), recovered a clade comprising the Caeciliidae, Typhlonectidae and Scolecomorphidae, a group informally known as the advanced (Nussbaum, 1991) or higher (San Mauro et al., 2004) caecilians. In their analyses, Uraeotyphlidae, Ichthyophiidae and Rhinatrematidae were recovered as successively more distant outgroups to the higher caecilians. Wilkinson and Nussbaum (1996) and Wilkinson (1997) found strong support for a sister group relationship of Ichthyophiidae and Uraeotyphlidae (=Diatria, Wilkinson and Nussbaum, 2006), which together form the sister group to higher caecilians (=Teresomata, Wilkinson and Nussbaum, 2006). A monophyletic Diatria have been recovered as the sister group to

Teresomata in all subsequent molecular studies of caecilian relationships (e.g. Wilkinson et al., 2002, 2003; San Mauro et al., 2004; Frost et al., 2006; Roelants et al., 2007). Recently, Frost et al (2006) synonymized Uraeotyphlidae with Ichthyophiidae based on the apparent paraphyly of *Ichthyophis* with regard to *Uraeotyphlus* (Gower et al., 2002; Frost et al., 2006). They further synonymized both the Scolecomorphidae and Typhlonectidae with the Caeciliidae because of the paraphyly of the latter regarding the former two groups. However, while the paraphyly of Caeciliidae with regards to Typhlonectidae has long been recognized (e.g. Nussbaum, 1979; Hedges et al., 1993; Wilkinson, 1997; Wilkinson et al., 2002; Wilkinson et al., 2003), the paraphyly of Ichthyophiidae with regard to Uraeotyphlidae (Gower et al., 2002; Frost et al., 2006) has not been universally demonstrated (see Roelants et al., 2007) and merits further investigation. Further uncertain is the position of the Scolecomorphidae, which might be either basal to Caeciliidae plus Typhlonectidae (Roelants et al., 2007) or within Caeciliidae (Wilkinson et al., 2003; Frost et al., 2006). Here I follow the taxonomy of Wilkinson and Nussbaum (2006).

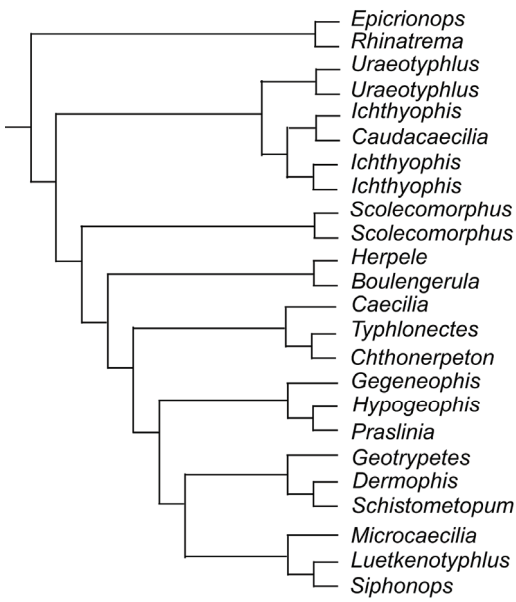

Fig. 3. Phylogenetic relationships of caecilians according to Roelants et al. (2007).

Since Nussbaum (1979) presented the first numerical analysis of caecilian intrarelationships, several studies have addressed this issue focussing on either larger scale relationships (e.g. Hay et al., 1995; Hedges and Maxson, 1993; Hedges et al., 1993; Frost et al., 2006; Roelants et al. 2007), intrafamilial or intrageneric relationships (e.g. Straub, 1985; Wilkinson and Nussbaum, 1999; Gower et al., 2002) or certain geographic areas (e.g. Hass et al., 1993; Gower et al., 2002; Wilkinson et al., 2002, 2003; Wake et al., 2004). Most of these studies are particularly interesting with regard to the relationships within the Caeciliidae, which is by far the largest and most diverse group in terms of ecology, morphological differentiation or life-history (Himstedt, 1996; Wilkinson and Nussbaum, 2006). While the position of some taxa like *Siphonops* is variable in several analyses (e.g. Wilkinson et al., 2003; Frost et al., 2006; Roelants et al., 2007), other relationships are consistently recovered in various analyses using different datasets, which lends some confidence to these results. Among these are the monophyly of the Seychellean caeciliids (e.g. Hass et al., 1993; Wilkinson et al., 2003), the sister group relationship of the Seychellean clade and *Gegeneophis* (e.g. Wilkinson et al., 2003; Roelants et al., 2007), the grouping of *Herpele* and *Boulengerula*, though deeply divergent, (Wilkinson et al., 2003; Frost et al., 2006; Roelants et al., 2007), and the sister group relationship of *Caecilia* and Typhlonectidae that renders the Caeciliidae paraphylectic (Hedges et al., 1993; Wilkinson et al., 2003; Frost et al., 2006; Roelants et al., 2007). Figure 3 shows the most recent phylogeny recovered by Roelants et al. (2007) using a large molecular data set and the most diverse sampling to date.

Skull morphology of caecilians

All caecilian species possess a heavily ossified skull (see Fig. 4 A, B) that is in stark contrast to the loftier and almost fragile skull morphologies seen especially in most frogs (Trueb, 1993). The fenestration of the cheek region is strongly reduced (a condition known as zygokrotaphy) and most species have a temporal region that is completely covered by bone (stegokrotaphy). Even the orbit is be completely covered by bone in some species with greatly reduced eyes. The

peculiar morphology of the caecilian skull is considered by virtually all authors to be an adaptation to a burrowing lifestyle (e.g. Müller, 1831b; Peters, 1880; Marcus et al., 1933; Taylor, 1969a; Duellman and Trueb, 1986; Nussbaum, 1998; Nussbaum and Wilkinson, 1989). There appears to be a correlation between the degree of the reduction of the temporal gap or the recession of the mouth and the degree of burrowing ability and subterranean lifestyle (Ramaswami, 1941; Taylor, 1969a; Teodecki et al., 1998; Gower et al., 2004).

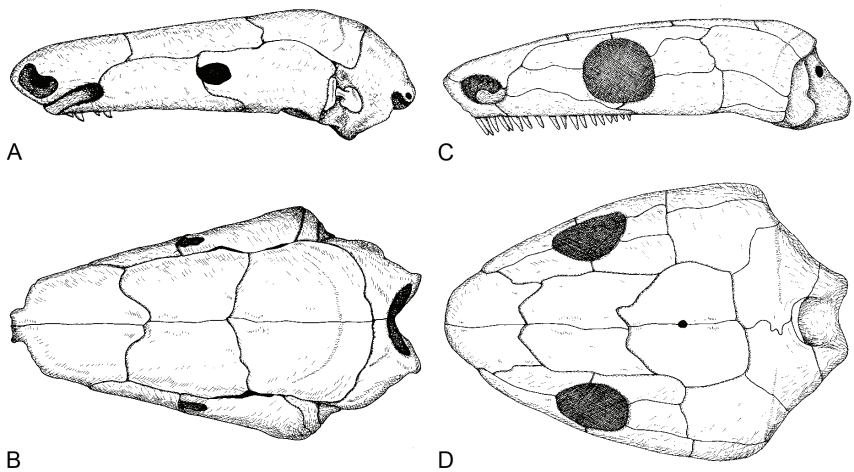

Fig. 4. Lateral (A) and dorsal (B) view of the skull of the adult caecilian *Hypogeophis rostratus* (modified from Taylor, 1969a). Lateral (C) and dorsal (D) view of the skull of the lepospondyl microsaur *Rhynchonkos* (from Carroll and Currie, 19675).

Caecilian skull morphology has been of considerable interest to many morphologists since the early days of comparative morphology, and a surprising amount of literature on adult skull morphology is available (see Straub, 1985 for a detailed list of the older literature on caecilian skull morphology and Wake, 2003 for a more recent summary). Dugés (1835) was among the first to provide a detailed examination of the caecilian skull. He and others (e.g. Wiedersheim, 1879) proposed that some of the large ossifications of the adult caecilian skull, such as the os basale (comprising the posterior part of the endocranium including the otic capsules as well as the floor of the braincase) are likely the product of a fusion of several individual ossifications during ontogeny. The composite nature of several of the bones that form the caecilian cranium was later corroborated by

ontogenetic information (e.g. Peter, 1898; Eifertinger, 1933; Marcus et al., 1935). In addition to the aforementioned os basale, all adult caecilians have the lower jaw elements fused into two large bones, the pseudodentary and pseudoangular, and the maxilla and palatine are fused to form the maxillopalatine in the adult cranium. The sphenethmoid has further been thought to be comprised of several individual endocranial ossifications (Marcus et al. 1935, Wake and Hanken, 1982; Müller et al., 2005) although there is controversy regarding how many elements are actually involved. These so-called compound bones are characteristic of the caecilian cranium and there is a phylogenetic trend towards an increased fusion of bones within the group. Rhinatrematids, ichthyophiids, uraeotyphlids and scolecomorphids are all characterized by separate nasal, premaxilla and septomaxilla bones, whereas the anterior snout region of caeciliids and typhlonectids is formed by the paired nasopremaxillae (Marcus et al., 1933; Taylor 1969a). Rhinatrematids, ichthyophiids, uraeotyphlids and scolecomorphids retain separate prefrontal bones that are absent in caeciliids and typhlonectids. However, a small element in a similar position but thought to be a lacrimal is incorporated into the maxillopalatine in caeciliids (Marcus et al., 1935; Müller et al., 2005). Ichthyophiids and uraeotyphlids further possess a circumorbital thought to be homologous with a postfrontal (Trueb, 1993).

A further phylogenetically variable feature is the degree of the closure of the cheek region (Taylor, 1969a; Trueb, 1993). Several taxa (rhinatrematids, uraeotyphlids, typhlonectids, *Scolecomorphus*, *Geotrypetes*) have zygokrotaphic skulls, in which a temporal gap separates the squamosal and parietal, whereas the remaining taxa have a stegokrotaphic or at least weakly stegokrotaphic (some ontogenetic variation is bound to occur) skull that has a completely closed cheek region. Zygokrotaphy in rhinatrematids is different from that of the remaining zygokrotaphic taxa in that the primary jaw adductor musculature extends onto the dorsal side of the skull (Nussbaum, 1983). Most authorities consider zygokrotaphy as exhibited by rhinatrematids to be the ancestral condition for Recent caecilians with stegokrotaphy being secondarily evolved (e.g. Sarasin and Sarasin, 1887-1890; Peter, 1898; de Beer, 1937; Ramaswami, 1941; Nussbaum,

1977, 1983; Wake and Hanken, 1982), although some have argued for stegokrotaphy as the plesiomorphic condition (e.g. Marcus et al., 1933, 1935; Carroll and Currie, 1975). The reconstruction of the ancestral condition is complicated by the recent discovery of the putative stem line caecilian *Eocaecilia micropodia* that has a clearly stegokrotaphic skull, which has been considered as a decisive support for stegokrotaphy being the ancestral condition in caecilians (Jenkins and Walsh, 1993; Carroll, 2000).

Development of the caecilian skull

Due to the paucity of suitable material, very few studies exist that have described the development of the skull in caecilians in any detail. Most studies of caecilian skull development, especially in the older literature, focus on either specific anatomical regions and do not present a coherent overview of cranial development (e.g. Peter, 1898; Jurgens, 1971; Reiss, 1996) or are based on single or few specimens (e.g. Winslow, 1898; Marcus et al., 1935; Ramaswami, 1948). Only relatively recently have larger developmental series been examined and described in detail (Wake and Hanken, 1982; Müller et al., 2005). Wake et al. (1985) further described skull development in *Typhlonectes compressicauda* but focused only on the regression of the cartilage associated with increasing ossification during development. Interestingly, skull development and life-history seem to be linked as indicated by different ossification sequences in direct-developing and viviparous species (Wake and Hanken, 1982; Müller et al., 2005), but this is based on very sparse taxon sampling.

The most influential early work on caecilian skull development was the account of Marcus et al. (1935) on the development of the skull in *Hypogeophis rostratus* and *Grandisonia alternans*, which was at this time considered to be a species of *Hypogeophis*. In this and previous papers (e.g. Eifertinger, 1933; Marcus, 1933) Marcus and co-authors described the skull and lower jaw as being composed of several individual bones that fuse during ontogeny to form the compound bones of the adult skull. Although the composite nature of several of the adult skull bones had already been demonstrated by Peter (1898), the high

number of separate ossification centres reported by Marcus et al. (1935), and thus the high extent of fusion occurring in the caecilian cranium, aroused much interest and was highly influential in the debate concerning their systematic position (e.g. de Beer, 1937). More importantly, the results of Marcus et al. (1935) were often seen as representing the standard in cranial development of Gymnophiona. Accordingly, in many subsequent studies on caecilian skull morphology (e.g. Ramaswami 1948; Brand 1956; Visser 1963) authors commented on several fused bones, the presence of which was more assumed than observed. Subsequent workers, however, pointed out inconsistencies (Brand, 1956) and, more recently, incongruence (Wake and Hanken, 1982; Müller et al., 2005) with results reported by Marcus and students.

Caecilian life-history

Despite being a comparatively small group, caecilians exhibit all the major life history modes seen in frogs and salamanders: oviparity with a free-living larva (e.g. Sarasin and Sarasin, 1887-1890), oviparity with direct development (e.g. Brauer, 1897) and viviparity (e.g. Peters, 1874). Fertilization is internal in caecilians via the phallus, a copulatory organ derived from parts of the hindgut and unique within lissamphibians (Gower and Wilkinson, 2002). Eggs of oviparous species are usually large (Exbrayat, 2006) and females guard their eggs where known (e.g. Sarasin and Sarasin, 1887-1890; Brauer, 1897; Sanderson, 1937, Kupfer et al., 2004, 2006). Species with free-living larvae undergo a metamorphosis to attain the adult-like morphology, although very little information is available on caecilian metamorphosis in general (e.g. Fox, 1987; Fritzsch, 1990; see also Wake, 2006). Viviparous species are characterized by smaller eggs and various forms of maternally provided, intraoviductal nutrition (Wake, 1977). Several recent studies have further drawn attention to a previously unsuspected degree of reproductive diversity among caecilians. O'Reilly et al. (1998) described altricial young in the viviparous *Geotrypetes seraphini* and Loader et al. (2003) described a young *Scolecomorphus vittatus* and suggested that two different modes of viviparity occur in caecilians. One mode is

characterized by large young that are independent at birth whereas species of the second mode give birth to altricial young that receive some form of extended post-parturition parental care. Kupfer et al. (2006) described altricial young in a direct developing caecilian, *Boulengerula taitanus* from Kenya that feed on the specially modified skin of their mother and is further characterized by so-called foetal teeth, and suggested that this life-history might have been a plausible intermediate step in the evolution of viviparity in caecilians.

Although the reproductive mode is known for fewer than half of the nominal species (Wake, 2006), interpolation from species with known reproductive mode to congeners provides a reasonable estimate of the distribution of reproductive modes in caecilians. The majority of species appear to be oviparous with direct development, followed by oviparity with a free-living larva (Wilkinson and Nussbaum, 1998). Viviparity is found in approximately one fifth of all species, which is a considerably higher proportion than in frogs or salamanders, where true viviparity is exceedingly rare (Wake, 1977). The phylogenetic distribution of the main reproductive modes in caecilians suggests several instances of independent evolution of viviparity and possibly direct development (Wilkinson et al. 2003; see Chapters 4 and 8). Life-history is further likely to have an impact on skull development and the limited available information seems to indicate differences between viviparous and non-viviparous species at least. Wake and Hanken (1982) discovered an altered sequence of skull ossification in the viviparous *Dermophis mexicanus*, in which bones involved in jaw articulation develop early as compared to non-viviparous species, and attributed this to active intraoviductal feeding early during ontogeny.

Aims of this thesis

The aims of this thesis are threefold: firstly to investigate the development of the caecilian skull in order to address the inconsistencies between earlier investigations (e.g. Marcus et al., 1935) on caecilian skull development and more recent studies (Wake and Hanken, 1982; Müller et al., 2005), secondly to investigate the metamorphosis of the caecilian skull and its associated

musculature in species with free-living aquatic larvae in order to analyse and document the changes occurring during metamorphosis and their bearing on interpretations of caecilian skull evolution, and thirdly to investigate the influence of different reproductive modes on the development of the skull. Chapter 1 provides a general introduction to caecilian amphibians and their inter- and intrarelationships. It further highlights the particularities of the caecilian skull compared to other recent and fossil amphibians, and summarizes current knowledge about its development, and also provides a short introduction to caecilian life-history. Chapter 2 investigates the development of the skull, lower jaw and hyobranchial skeleton of *Hypogeophis rostratus*, which was the subject of extensive study by the Marcus school during the 1920s and 1930s. Marcus and his students reported a surprisingly high number of separate ossifications that occur during the development of the skull in this and a closely related species. Their results proved very influential in the debate about the phylogenetic position of caecilians and the evolution of their unique skull morphology, although more recent studies (Wake and Hanken, 1982; Müller et al., 2005) have failed to confirm several of the observations of Marcus and his students. Chapter 3 investigates the morphology of larvae and adults of all taxa known to have a biphasic life-history. The more basal branching caecilian taxa Rhinatrematidae, Ichthyophiidae and Uraeotyphlidae (as well as several caeciliids) are characterised by oviparity with a free-living, usually aquatic larva that subsequently undergoes a metamorphosis to attain the adult-like morphology. Metamorphosis in caecilians is very poorly known, with no information being available for many of the taxa concerned. The metamorphic changes are analysed and their bearing on the ground pattern of the cranium of caecilians addressed. The following three chapters focus on aspects of the developmental diversity of caecilians. Chapter 4 compares the posthatching development of the skull in two different direct-developing species with that of a species with free-living larvae. Chapter 5 describes the remarkable form of post-hatching parental care in *Boulengerula taitanus* that involves juveniles feeding on their mother's own skin, while Chapter 6 describes and analyses the unusual morphology of the head in

foetal and juvenile *Scolecomorphus kirkii*. Chapter 7 provides a synopsis of the key findings of this thesis and provides a look forward to the future of studies on caecilian development.

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CHAPTER 2

Ontogeny of the skull, lower jaw and hyobranchial skeleton of *Hypogeophis rostratus* (Amphibia: Gymnophiona: Caeciliidae) revisited

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ABSTRACT Few detailed descriptions of the development of the head skeleton in caecilian amphibians are available. One of those is the work of Marcus and students (e.g., Gehwolf, 1923; Marcus, 1933; Marcus et al., 1935) on the morphology and development of the skull, lower jaw and hyobranchial skeleton in the Seychellean caeciliids *Hypogeophis rostratus* and *Grandisonia alternans*. These workers described a high number of individual ossifications that fuse during ontogeny to form the adult skull. Although later studies have doubted the generality of those observations, the work of Marcus and his students has been hugely influential in subsequent studies of caecilian skull morphology and amphibian evolution. Based on new observations on an ontogenetic series of 32 sectioned and cleared and stained specimens, ranging from the beginning of chondrification to the adult, the development of the skull, lower jaw and hyobranchial skeleton of *H. rostratus* are described. The new results are largely incompatible with those of Marcus and students and no evidence for several of the reported ossifications, including supra-, infra- and basioccipital, epiotic, pleurosphenoid, preethmoid, posterior vomer, prepalatine, quadratojugal, postparietal, second coronoid, supraangular and complementare, is found. It is argued that most of Marcus et al.'s reports of non-existent ossifications are based on false phylogenetic preconception, misinterpretation of the observed morphology and technical error. Data on the ossification sequence of the skull and lower jaw in *H. rostratus* are provided and briefly compared to published information on *Dermophis mexicanus* and *Gegeneophis ramaswamii*.

INTRODUCTION

At a time when biosciences as a whole are a fast moving field, morphology and morphological systematics draw both from the most current studies using advanced analytical techniques, as well as from studies that sometimes date back as far as the 19th century. In some cases, such as rare and seldom studied animals for which new material is limited, old accounts often represent the only source of primary morphological data. These, however, as with almost all scientific studies, are the products of their time and are often as much a reflection of contemporary trends as they are documentations of the observed morphology. One possible case in point concerns the skull morphology of caecilian amphibians, where new studies (Wake and Hanken, 1982; Müller et al., 2005) have produced results largely incongruent with earlier studies of skull development (e.g., Marcus et al., 1935).

Caecilians (or Gymnophiona), frogs and salamanders constitute the three extant clades of the Amphibia. Caecilians comprise about 170 named species in six families (Wilkinson and Nussbaum, 2005). They are the least known, major living tetrapod clade, which is probably mainly due to their largely subterranean habits and confinement to parts of the wet and seasonal tropics of South and Middle America, Africa and Asia. The first extensive comparative studies of caecilian morphology were undertaken by Wiedersheim (1879) and Peters (1880). Taylor (1969b) provided brief descriptions of adult skull morphology for a broad range of taxa and, most recently, Wake (2003) reviewed and summarized known adult skull morphology of all caecilian genera. However, most studies on caecilian morphology have been restricted to investigations on adult material and were usually carried out on small samples (e.g., Brand, 1956). This led to uncertainties about bone homologies because the heavily ossified, burrowing-adapted caecilian skulls are highly modified compared to the skulls of other amphibians.

Little ontogenetic information about caecilians was available when Marcus et al. (1935) published a lengthy account on the development of the skull in what were then thought to be two species of *Hypogeophis*. Based on their observations

of a very limited number of three ontogenetic stages (early and late embryo and juvenile), they described a high number of individual bones that occur during the development of the skull. These bones were reported to fuse during ontogeny to form a highly compact adult skull that is made up of a relatively small number of bones, like the os basale, which is comprised of the dermal parasphenoid and most of the posterior part of the neurocranium. The formation of the large compound bones from individual ossifications had been already postulated by early students of caecilian skull morphology (Dugès, 1834; Wiedersheim, 1879), and was later corroborated by Peter (1898). However, the unusually high number of separate ossification centers reported by Marcus et al. (1935), aroused much interest (de Beer, 1937) and featured prominently in the still unsettled debate concerning the systematic relationships of living amphibians (Schoch and Milner, 2004 for most recent review). More importantly, because of the general lack of developmental studies in caecilians, the results of Marcus et al. (1935) were seen as representing the standard in caecilian cranial development. Several authors of subsequent studies on caecilian skull morphology (e.g. Ramaswami, 1948; Brand, 1956; Visser, 1963) commented on presumably fused bones, whose presence was assumed rather than actually observed. Some of the results of Marcus et al. (1935) were at least occasionally doubted (Brand, 1956) but were nonetheless largely accepted until Wake and Hanken's (1982) study of skull development in *Dermophis mexicanus*, a viviparous Central American caeciliid, in which they were unable to confirm several ossifications (e.g. basi- and supraoccipital, pleurosphenoid, postorbital, quadratojugal) reported by Marcus et al. (1935).

Recently, Müller et al. (2005) investigated the development of the skull in the *Gegeneophis ramaswamii*, an Indian direct-developing caeciliid more closely related to the Seychellean caecilian radiation comprising *Hypogeophis rostratus* and *Grandisonia* (Hass et al., 1993; Wilkinson et al., 2002) than *Dermophis mexicanus*. They too, found no evidence for many of the ossifications reported by Marcus et al. (1935) that Wake and Hanken (1982) did not find in *D. mexicanus*, which raised further doubt about the validity of many of Marcus et al.'s observations. To clarify these conflicting observations I analyzed a nearly

complete ontogenetic series of *H. rostratus* with regard to the ossifications, their homology, and their sequence of appearance.

MATERIALS AND METHODS

The material used in this study was collected by A. Brauer in 1896 in the Seychelles. The material is deposited in the Museum für Naturkunde, Berlin (ZMB) and also represents the source for the studies by H. Marcus and co-workers (e.g., Marcus, 1909:105, 110). I studied an ontogenetic series of embryos, juveniles and adults of *Hypogeophis rostratus* (Appendix). Embryos selected for clearing and staining were double stained for bone and cartilage using a slightly modified protocol based on Taylor and Van Dyke (1985). Specimens selected for serial sections were decalcified, embedded using a Shandon Hypercenter XP tissue processor, sectioned at 8 µm using a Leica SW 2000R microtome equipped with Feather N35H disposable blades and sections stained with azocarmine-red and anilin-blue (AZAN) following standard procedures (Romeis, 1989). For scanning electron microscopy (SEM), the skull of a cleared and stained juvenile was partly disarticulated and, using tweezers, the bones were gently freed from adhering fibrous tissue. Glycerin was washed out in ethanol and the bones air-dried, mounted, and sputter coated with gold-palladium. Cleared and stained specimens were observed under a Nikon SMZ-U stereomicroscope equipped with a camera lucida and a digital camera (Nikon Coolpix 995) or a Zeiss DR stereomicroscope. Photos of cleared and stained specimens were taken with a Zeiss Tessovar with a Nikon Coolpix 995 digital camera attached. Serial sections were observed under a Nikon Eclipse E600 microscope and SEM prepared specimens observed and photographed under a Hitachi S2500 SEM with a digital image capture system.

The overall preservation of the material was generally very good, given the long time in storage. Some of the cleared and stained specimens did not or not completely retain the alizarin red bone stain. This was particularly the case in earlier embryos. These were observed under indirect illumination (e.g. dark field),

under which developing and unstained or decalcified bone appears as a light structure (Haas, 1996). In the serial sections, bones and other elements were assessed based on their histological appearance rather than staining. This pertains especially to the distinction between cartilage, precartilage and mesenchyme in the hyobranchial skeleton. As Cartilage I recognized tissue characterized by the expression of cartilage extracellular matrix (ECM). Precartilage is an aggregation of densely packed nuclei that resemble those of cartilage but without visible cartilage ECM, whereas mesenchyme is a more diffuse yet recognizable aggregation of cells. Although useful as descriptors, these distinctions are somewhat arbitrary as they pertain to certain sections of a developmental continuum. I have therefore tried to avoid any over-interpretation based on these structures

In the description of the development of the skull, I distinguish between endoskeletal bone, dermal bone and membrane bone (following Patterson, 1977). Endoskeletal bone is bone that forms by peri- or endochondral ossification of a cartilaginous precursor, such as the exoccipital, which forms as a perichondral ossification of the cartilaginous exoccipital arch. Dermal bone develops without a cartilaginous precursor and has no connection to an endoskeletal element. Typical dermal bones are nasal and maxilla. Membrane bone is a form of bone that ossifies without a cartilaginous precursor but is phylogenetically part of the endocranium. All membrane bones in *Hypogeophis rotratus* and other caecilians investigated (see Müller et al., 2005) develop as a laminar outgrowth from endoskeletal bone, such as the dorsomedial outgrowth of membrane bone from the ossified otic capsule, above the foramen magnum. In all instances covered here, membrane bone extends from, and is always connected to, endochondral bone. For a reference to the morphology of the endocranium discussed in here, see Figure 2E.

To facilitate comparison with published accounts of development in *Hypogeophis rostratus*, embryos were staged according to Brauer (1899). Brauer's description of development in *Hypogeophis* is more of an overview, rather than a staging table in a modern sense, where development is divided into

discrete intervals defined by morphological, physiological, and behavioral markers (e.g., Nieuwkoop and Faber, 1967; Bartsch et al., 1997). Brauer's detailed figures and descriptions, however, were subsequently referred to as stages (e.g., Marcus, 1909:111; Eifertinger, 1933) and can be used as such. The published staging tables for the biphasic *Ichthyophis kohtaoensis* (Dünker et al., 2000) and viviparous *Typhlonectes compressicauda* (Sammouri et al., 1990) were inadequate for the description of development in *H. rostratus*, as several of the stage-defining characters (e.g., development of lateral line organs in *I. kohtaoensis*, formation of the sack-like gills in *T. compressicauda*) are not expressed in the direct developing *H. rostratus.* When comparing specimens to Brauer's (1899) account, I established approximate correspondences with his figured 'stages' based on features such as the development of the external gills, head flexure, and the amount of yolk. Marcus and co-workers staged their material in a similar way, and their specimens were found to match the newly staged material well in terms of skull development. I observed some intraspecific variation in skull development in my material, and although external development did not always reflect similar skull development in all specimens investigated, reference to Brauer's stages proved to be a better descriptor of development than a reference to size, which would have significantly increased the degree of intraspecific variation observed. In the following description, I describe skull development based on Brauer's 'stages' (abbreviated BS herein), as reference to BS is preferable to a comparison based on size, which is neither a reliable indicator of developmental progress nor a useful facilitator of interspecific comparisons.

Many caecilian species are fairly poorly defined (Nussbaum and Wilkinson, 1989; Gower and Wilkinson, 2005). One exception is the caecilians of the Seychelles archipelago (*Grandisonia alternans*, *G. brevis*, *G. larvata*, *G. seychellensis*, *Hypogeophis rostratus* and *Praslinia cooperi*), which are relatively well known taxonomically (Parker, 1958; Nussbaum, 1984; Wilkinson and Nussbaum, 2005). *Hypogeophis rostratus* is the most widespread species and occurs on every Seychellean island from which caecilians are known, and is often

the only caecilian species present (Nussbaum, 1984). Although all these populations are treated as belonging to *H. rostratus*, morphological divergences between several island populations have been recognized and led to the description of several subspecies (Parker, 1958; Taylor, 1968, 1969a). There is sufficient evidence that all of the specimens collected by Brauer (and used here) originate from Mahé and/or Silhouette, and thus would belong to the nominate subspecies *H. r. rostratus*. Here, however, I follow Nussbaum and Wilkinson (1989) in only recognizing nominal species, given that the biological and taxonomical meaning of subspecies is poorly defined.

RESULTS

Development of the skull

At BS 38 (the earliest developmental stage available) the chondrocranium is relatively incomplete. Chondrification proceeds from posterior to anterior. The occipital arch, together with the palatoquadrate, is most prominently developed but does not contact the otic capsule and parachordal cartilage (*cf.* Fig. 1A). The otic capsule, especially its medial wall, is only weakly chondrified, has a large fenestra ovalis, and is connected to the parachordal cartilage at its anterior and posterior ends, albeit via very weakly developed cartilaginous bars. A small, weakly chondrified, Y-shaped cartilaginous stapes is present at the anteroventral end of the fenestra ovalis. The palatoquadrate is a fairly large element and oriented dorsoventrally. In lateral view, its ventral half is slightly broader than its dorsal half. It further has a clearly discernible articular facet and a small pterygoid process, which is continuous with the main body of the palatoquadrate ventromedially. The parachordal cartilage is only weakly chondrified anterior to the otic capsule. The taenia marginalis shows the same degree of chondrification as the parachordal cartilage and extends from the anterodorsal end of the otic capsule to the level of the palatoquadrate. It is continuous with the otic capsule although the connection is narrow and almost unstained.

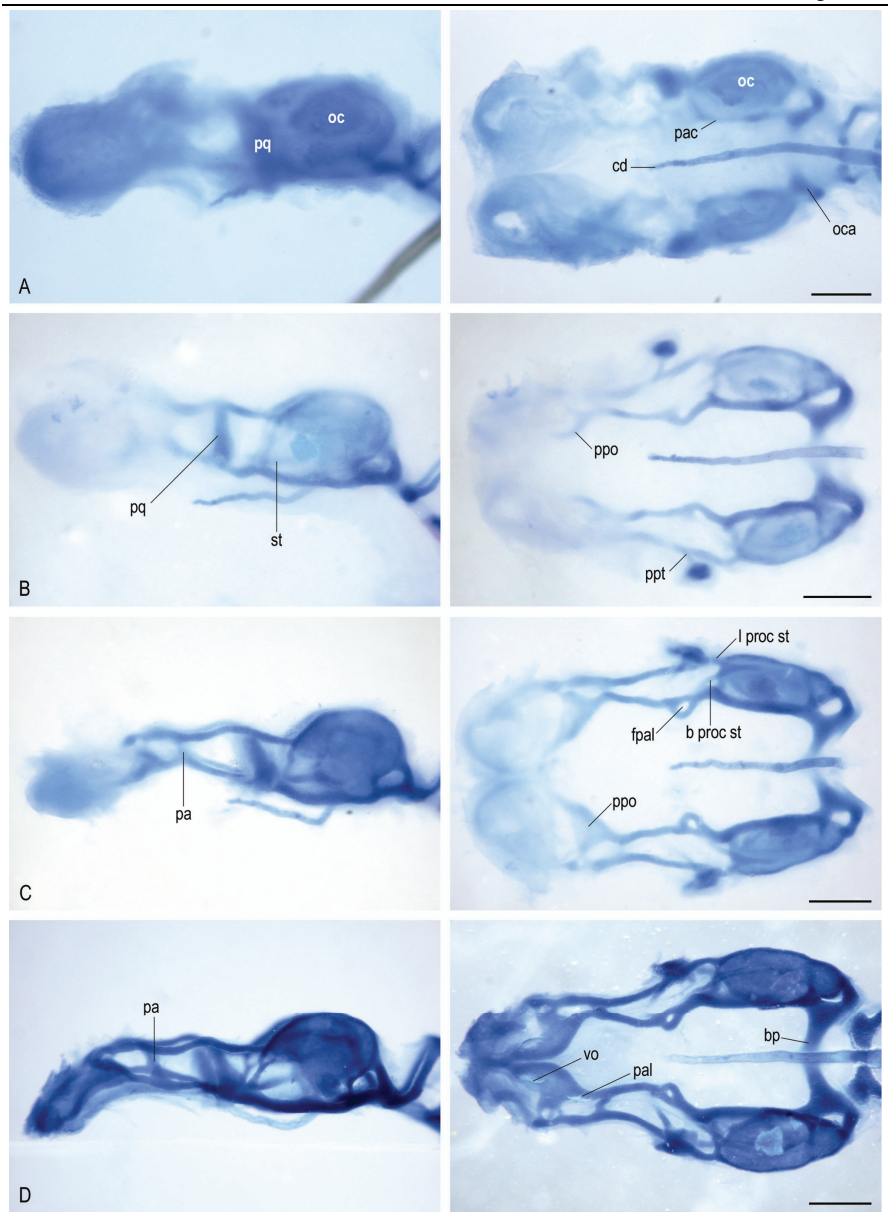

Fig. 1. Development of the skull in *Hypogeophis rostratus*; lateral views on the left and ventral views on the right. **A**: BS 38/39 (Hyro 26); note the unfused occipital arch (oca) and parachordal cartilage (pac). **B** BS 40 (Hyro 25). **C**: BS 40 (Hyro 27). **D**: BS 41/45 (Hyro 14); vomer (vo) and palatine (pal) are present but unstained because of decalcification/insufficient ossification. bp, basal plate; b proc st, basal process of the stapes; cd, chorda dorsalis; fpal, palatine foramen; l proc st, lateral process of the stapes; oc, otic capsule; pa, pila antotica; ppo, pila preoptica; ppt, pterygoid process of the palatoquadrate/quadrate; pq, palatoquadrate; st, stapes. Scale bar equals 0.5 mm.

Chondrocranial development has progressed little in a BS 38/39 embryo (Fig. 1A), however, chondrification of the otic capsules is more extensive and a basal plate is forming ventrally between the occipital arches. The parachordal cartilage remains weakly developed anterior to the otic capsule. The taenia marginalis is more chondrified and extends further rostrally than in the previous embryo. The connection to the otic capsule, however, is constricted and stained less intensely than the rest of the taenia marginalis.

The embryos of BS 40 available for study (Hyro1, sectioned; Hyro 25 and Hyro 27, cleared and stained) show some variation in the degree of skeletal development, as can be seen in Fig. 1B and C. A very weakly chondrified pila antotica is discernible just posterior to the position of the eye in Hyro 25 (*cf.* Fig. IB), connecting the taenia marginalis and parachordal cartilage. Anterior of the pilae antoticae, the trabecular cartilages bend inwards and converge towards the midline. Anterior of the pila antotica, anlagen of the trabecular cartilage, pila preoptica, and nasal capsule are just visible in Hyro 25. Hyro 27 shows a more advanced development of the trabecular cartilage, pila preoptica and nasal capsule (*cf.* Fig 1C). The occipital arch is fused with the otic capsule and parachordal cartilage. The basal plate is more chondrified than in the previous embryo although the part closest to the chorda dorsalis remains only faintly stained. The parachordal cartilage anterior to the otic capsule has a foramen for the palatine branch of the facial nerve. Also, the basal process of the stapes is beginning to fuse to the parachordal cartilage and the palatoquadrate is more anteriorly inclined than in the previous specimens. The sectioned specimen (Hyro 1) exhibits the same degree of chondrification as Hyro 27. In addition, small ossifications of vomer and palatine are present. The vomer forms as a simple blade anterolaterally of the convergence of the preoptic plates. The palatine consists of a simple sliver of bone, only half the size of the vomer, and forms just posterolaterally of the choana.

In embryos of BS 40 to BS 40/41 (Fig. 1D), most of the chondrocranium except the anterior nasal capsule is well developed, although the otic capsule remains weakly chondrified ventrolaterally. Perichondral ossification is apparent

at the posterior part of the chondrocranium, where the exoccipital forms as a thin layer of bone sheathing the occipital arch. Ossification of the prootic is apparent in the anterodorsal part of the otic capsule and the posterior taenia marginalis. Anterior to the otic capsule, the parachordals are somewhat compressed dorsoventrally and curve gently inwards. A zone of weak chondrification is apparent just anterior to the otic capsule, at the point where the basal process of the stapes joins the parachordals (processus columello-trabecularis *sensu* Visser, 1963). From histological sections, this area consists of cell-rich cartilage and the parachordal and stapes are fused rather than in articulation. Just anteriorly is the foramen for the palatine nerve that pierces the parachordal. A laterally-directed, short cartilaginous process is anterior to the palatine foramen and will form the basipterygoid articulation from BS 45 onwards. Further anteriorly, the parachordal becomes more rounded and curves laterally towards the pila antotica. The pila antotica forms a well chondrified but slender bar. Anteriorly, trabecular cartilages are well developed and expand into a pair of broad and somewhat weakly chondrified pilae preopticae, which elongate anteriorly and eventually fuse to form the nasal septum that extends beyond the anterior copulae into the processus prenasalis. The nasal septum also forms a posterodorsally-directed process. Except for the nasal septum and oblique cartilage, most of the anterior and lateral parts of the nasal capsule remain only weakly chondrified. The stapes is a well-chondified, triradiate cartilage. The footplate is rod-like and sits within the widely open fenestra ovalis. Anterior to the fenestra ovalis, the stapes bifurcates and forms a short, anteriorly-expanded lateral process that articulates with an inconspicuous process at the posterior margin of the palatoquadrate, and a basal process that fused with the parachordal. The ventral half of the palatoquadrate, including the well-developed processus pterygoideus, is covered by a thin layer of perichondral bone. A thin, blade-like premaxilla is present anteriorly, ventral of the anterior copula. It is followed by more developed and larger vomer and palatine ossifications posteriorly. The vomer has enlarged and is crescent-shaped in ventral view. It consists of a dental lamina and a narrow palatine shelf that is growing towards the midline. The plate-like palatine has also

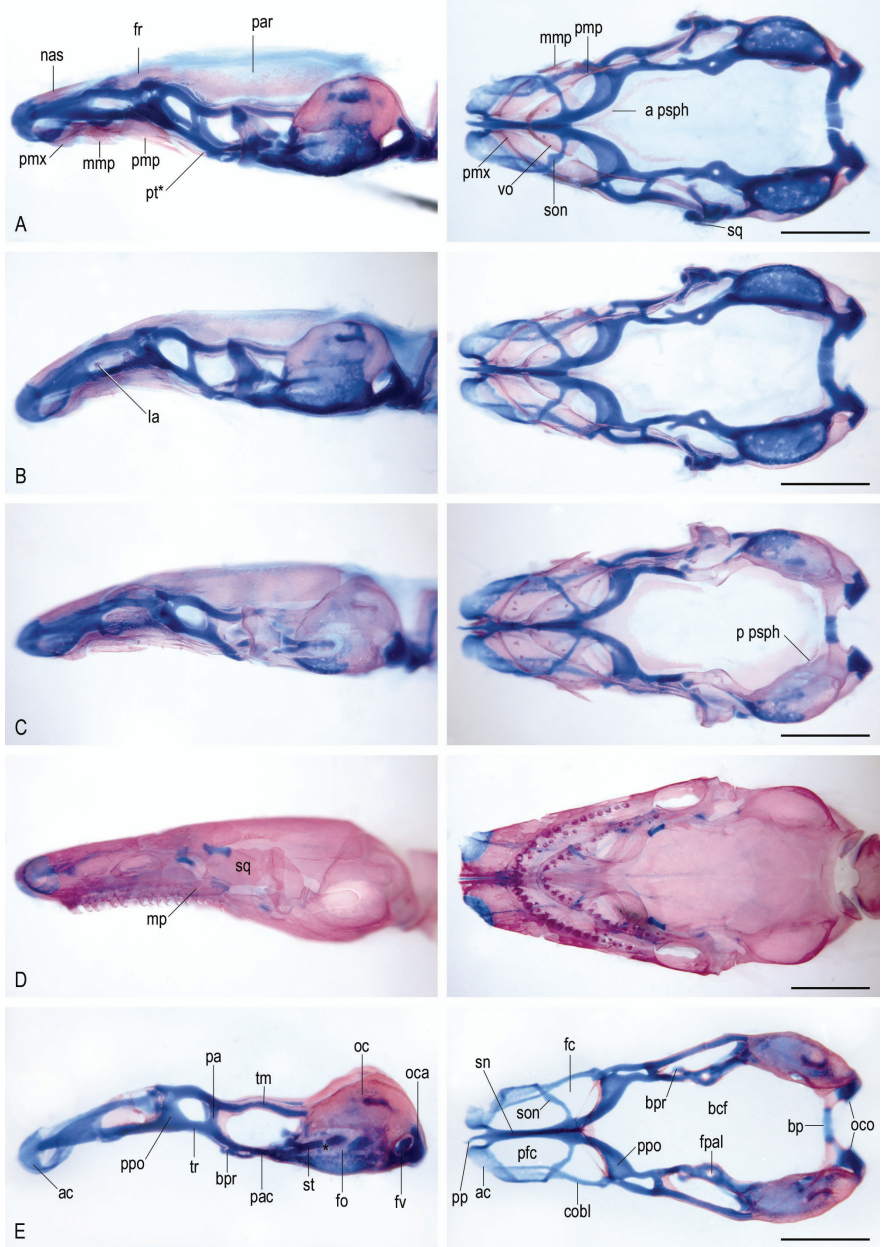
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Fig. 2. Development of the skull in *Hypogeophis rostratus*; lateral views on the left and ventral views on the right. **A**: BS 45 (Hyro 11). **B**: BS 46 (Hyro 19). **C**: BS 47 (Hyro 17). **D**: Juvenile (Hyro 24); the squamosal (sq) covers the cheek region and is in contact with the maxillopalatine (mp). **E**: BS 47 (Hyro 21); endocranium, the quadrate/palatoquadrate and all dermal elements are removed. a psph, anterior part of parasphenoid; ac, anterior copula; bfc basicranial fenestra; bp, basal plate; bpr, basal process; cobl, oblique cartilage; fc, choanal foramen; fpal, palatine foramen; fo, fenestra ovalis; fr, frontal; fv, vagus foramen; la, lacrimal; mmp, maxillary part of the maxillopalatine; nas, nasal; oc, otic capsule; oca, occipital arch; oco, occipital condyles; pa, pila antotica; par, parietal; pfc, prechoanal foramen; pmp, palatine part of the maxillopalatine; pmx, premaxillary; p psph, posterior part of parasphenoid; pac, parachordal cartilage; pp, prenasal process; ppo, pila preoptica; pt*, fused pterygoid; sn, septum nasi; son, solum nasi; st, stapes; tm, taenia marginalis; tr, trabecular cartilage; vo, vomer. * marks a damage due to handling. Scale bar equals 1 mm.

enlarged and its anterior half is twisted against its posterior half, giving it an hourglass-like shape if seen from ventral. Further posterior to the palatine is a long and thin, plate-like pterygoid (Fig. 3D) that stretches to the anterior tip of the pterygoid process of the palatoquadrate.

By late BS 41/45, ossification of the quadrate has started (Fig. 3E) with a thin layer of perichondral ossification surrounding the pterygoid process and the ventromedial and ventrolateral sides of the palatoquadrate cartilage. A small frontal is present as a small, narrow sheet of bone laterally, above the oblique cartilage. A parietal is present too as a relatively long sliver of bone above the taenia marginalis. A small, short plate-like maxilla is found laterally of the posterior half of the nasal capsule. The dermal pterygoid is fused to the perichondral ossification surrounding the pterygoid process of the quadrate.

The available BS 45 specimens do also show variation in the degree of skeletal development. In the least advanced embryo (Hyro 5), chondrocranial development has greatly progressed as compared to previous stages. The fenestra ovalis remains comparatively large and not yet filled by the stapes. The occipital arch is completely sheathed with perichondral bone, as are the posterior part and most of the medial wall of the otic capsule. A layer of perichondral bone also covers the anterodorsal surface of the otic capsule and posterior parachordals. The stapes, still unossified, tightly abuts the palatoquadrate. The connection between the stapes and the parachordal cartilage is robust and well chondrified, fusing the stapes to the parachordals (Fig. 3F). The palatoquadrate cartilage is partly replaced by the quadrate bone, and the dermal pterygoid is fused to the ossified

endoskeletal pterygoid process of the quadrate. The nasal capsule is fully formed and is composed of anterior copulae and well chondrified lateral walls. A cartilaginous roof is absent and the sola nasi consist of thin strands of cartilage (Cornu laterale *sensu* Marcus et al., 1935). The premaxilla has grown considerably and become crescent-shaped, with a distinct dental lamina. A maxilla is present as a simple, relatively deep but short, plate-like ossification lateral of the posterior nasal capsule. Vomer and palatine have also enlarged and their dental laminae are very distinct. The palatine shelf of the vomer is much enlarged and has a notch for the palatine branch of the facial nerve on its medial edge. The palatine has a large, vertically oriented lamina medial to the eye and lateral to the nasal epithelium. Posterior of the choana, a medially directed process has formed through the accretion of bone, which gives the palatine its characteristic Y-shape when viewed from ventral. One to two premaxillary, vomerine, and maxillary tooth crowns are present but not fused to their respective bones as their pedicels are not yet developed. The anterior part of the parasphenoid is starting to form as a V-shaped bone at the anterior edge of the basicranial fenestra. Frontal and parietal bones remain long and narrow plates of bone dorsolaterally, above the oblique cartilage and taenia marginalis respectively. In the most advanced BS 45 specimen (Hyro 11; Fig 2A), skeletal development has progressed further. The dorsal and medial part of the otic capsule is almost completely ossified, with cartilage mainly confined to the ventrolateral region. Membrane bone extends dorsomedially from the posterior dorsomedial margin of the otic capsules, forming the dorsal rim of the foramen magnum. The anterior footplate of the stapes is perichondrally ossified. A lamina of membrane bone, extending from the taenia marginalis, lies dorsally behind the pila antotica. A sphenethmoid ossification is present and consists of a median bony lamina anterior of the pilae preopticae (trabecular plates) that is continuous with perichondral ossification of the cartilaginous nasal septum. Laterally, thin lamellae of membrane bone, which are part of the sphenethmoid, form the anterior wall of the brain cavity. Small nasals are present and lie dorsomedially of the nasal capsule. The anterior parasphenoid extends caudally to the level of the

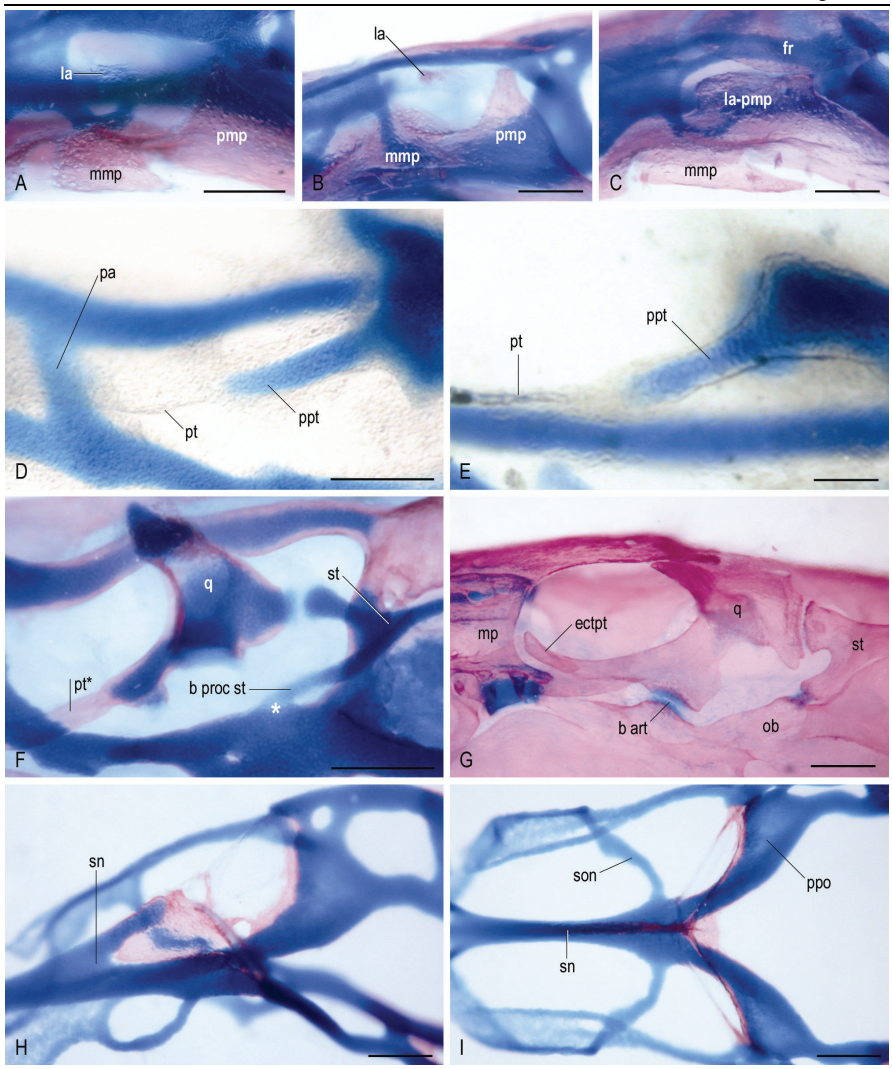

Fig. 3. Aspects of skull development in *Hypogeophis rostratus*. Close-up of the development of the lacrimal (la) from its first occurrence at BS 45 (**A**, lateral view; Hyro11), through BS 46 (**B**, slightly ventrolateral view; Hyro 19) and its eventual fusion to the palatine part of the maxillopalatine (pmp) at BS 47 (**C**, lateral view; Hyro 17). The free pterygoid (pt) in an embryo of BS 41 (**D**, ventrolateral view; Hyro 28) and BS 41/45 (**E**, lateral view; Hyro31); note the extensive perichondral ossification around the pterygoid process (ppt) and quadrate in **E**. Fusion of the basal process of the stapes (b proc st) is marked with an asterisk (*) and shown in **F** in a BS 45 embryo (Hyro 11). **G** Juvenile skull (Hyro 24) in ventral view, showing the ectopterygoid (ectpt) just posterior of the maxillopalatine (mp); note the cartilage covered articular facets of the basal articulation (b art) and the cartilaginous connection between the stapes (st) and os basale (ob). Dorsolateral (**H**) and lateral view (**I**) of the sphenethmoid ossification in a BS 47 embryo (Hyro 21). Additional abbreviations: mmp, maxillary part of the maxillopalatine; pa, pila antotica; ppo, pila preoptica; pt*, fused pterygoid; q, quadrate; sn, septum nasi; son, solum nasi. Scale bar equals 250 µm in all pictures.

basal process and has also developed an anterior directed process that extends rostrally between the vomers up to the palatine foramen of the vomer. Maxilla and palatine are fused to form the maxillopalatine. The maxillary part of the maxillopalatine, however, remains short. A few maxillary, premaxillary, vomerine, and palatine tooth crowns are present but not attached to their respective bones. The squamosal is present as a distinct, albeit small, elliptical bone lateral to the dorsal half of palatoquadrate. A small lacrimal bone is present above the maxilla, anterior to the orbital shelf of the palatine (Fig. 3A). The premaxilla has developed an alary process that extends dorsally between the anterior copula and the prenasal process. The vomer has completely encircled the palatine branch of the facial nerve. Frontal and parietal have approached each other more closely and have also grown further towards the dorsal midline. The leading, medial edges of the frontal and especially the parietal are less intensely stained than the lateral parts and show a reticulated staining pattern.

At BS 46 (Fig. 2B) endocranial ossification has progressed further. Most of the endocranium posterior to the pila antotica is covered with a layer of perichondral bone. The lamina of membrane bone behind the pila antotica has enlarged, extends onto the pila antotica, and is fused ventrally to the perichondral bone layer around the parachordal. In the ethmoidal region, the sphenethmoid has expanded further and forms a well-developed bony nasal septum. The bony nasal septum is continuous with the perichondral ossification around the cartilaginous nasal septum and the perichondral ossification of the pila preoptica (*cf.* Fig. 3H,I).

The palatoquadrate is completely covered by a layer of perichondral bone, i.e. the quadrate bone. The stapes is also completely sheathed with a thin layer of perichondral bone, except for the sites of articulation with the quadrate and parachordal. Most dermal bones have expanded, particularly the nasal, frontal and parietal. Whereas the frontal and parietal are growing in a lateral to medial direction, the nasal is growing from medial to lateral. The squamosal remains comparatively small and still has not grown out to cover the cheek region. A small dermal ectopterygoid ossification (*cf.* Fig. 3G) is present lateral to the anterior pterygoid process and posterolaterally to the maxillopalatine. Splints of

bone are present in the anlagen of the posterior parasphenoid, anterolaterally of the basal plate.

At BS 47 (Fig. 2C), most of the posterior part of the endocranium up to the pila antotica is ossified, with small remnants of cartilage confined to the occipital arch, the medial part of the basal plate, and the anteroventral wall of the otic capsule. The footplate of the stapes has broadened and the stapes is completely covered by perichondral bone except for the articulations with the quadrate and the former parachordal, which has been integrated into the endocranial part of the os basale. The sphenethmoid ossification has expanded and replaced the cartilage in the centre of the pila preoptica. The posterior and anterior parts of the parasphenoid have enlarged and additional small splints of bone are present just posteromedial of the palatine foramen. The anterior part of the parasphenoid completely fills the anterior basicranial fenestra, between the preoptic plates. The lacrimal is fused posteriorly to the orbital shelf of the palatine part of the maxillopalatine (Fig. 3C). Nasals and premaxillae are fused between the anterior copulae. The squamosal remains comparatively small and covers only the anterodorsal part of the quadrate laterally. In the most advanced BS 47 specimen (Hyro 7), the separate parts of the parasphenoid have fused and expanded and this bone covers the entire basicranial fenestra, apart from a relatively small, medial area at the level of the quadrate, where ossification is poor.

In juveniles (Figs. 2D, 3G), only part of the orbital and trabecular cartilages, part of the orbitonasal orifice, the prenasal process, the basal articulation, and the connection of stapes and os basale remain cartilaginous. The posterior endocranium and parasphenoid are fused and form the os basale. The skull has a morphology comparable to adult specimens in that the maxillopalatine has grown caudally to contact the squamosal, which has grown rostrally and covers the cheek region laterally. Both bones do also form the orbit and completely encircle the eye. The pedicels of most teeth are fused to their respective bones, thus anchoring the tooth crowns, and the dental arcades have the same extent and shape as in the adult cranium. Most of the dermal bones, however, are not as tightly sutured as in the adult skull. The membrane bone that

forms the upper margin of the foramen magnum is still not fully developed, leaving a midline gap.

Development of the lower jaw

At BS 38, Meckel's cartilage is a well chondrified but simple rod. The anterior tips are separated by a fairly broad gap. Posteriorly, the retroarticular process of Meckel's cartilage does extends only a short distance beyond the jaw articulation. Meckel's cartilage becomes lyre-shaped (in ventral view) at BS 38/39 and a broad symphyseal area has formed at the anterior midline. A processus condyloideus is present and articulates with the palatoquadrate, and the processus retroarticularis is prominently developed and extends well beyond the jaw articulation. The anlage of a dentary, the first ossification to appear in the skull, is present laterally at the anterior tip of, and in very close proximity to, Meckel's cartilage.

The dentary has enlarged in a BS 40 embryo, and is continuous with perichondral ossification that has developed at the anterior tip of Meckel's cartilage. An angular is present along the ventral side of Meckel's cartilage. It is a long, rod-like bone that is almost rectangular in transverse sections. A thin, plate-like bone covers the lingual side of Meckel's cartilage anterior of the jaw articulation. In the youngest embryo where it is visible (Hyro 1, BS40), this bone has a very narrow connection to the angular below the articular surface of the lower jaw.

At BS 40/41, the dentary has expanded further and the angular is fused to the lingual bony lamina, except for a very large medial foramen through which the mandibular branch of the trigeminal nerve passes.

At BS 45, the anterior tip of Meckel's cartilage is extensively endochondrally and perichondrally ossified. Most of its labial and ventral side is covered by the dentary, which extends back to the level of the jaw articulation. A well-ossified coronoid is present and fused to the perichondral ossification at the tip of Meckel's cartilage. Dentary tooth crowns appear first, followed shortly after by coronoid tooth crowns. Neither set of teeth is attached to the bones yet. The angular has greatly expanded and covers most of the lingual and ventral side

of Meckels cartilage. It is fused to the perichondral articular close to the jaw articulation, and the retroarticular process is completely perichondrally ossified.

The dentary, angular, and coronoid are slightly larger at BS 46, and additional dentary tooth crowns are present. By BS 47, Meckel's cartilage has been replaced by bone in the anterior part of the lower jaw and also the area of the jaw articulation. The retroarticular process is heavily perichondrally ossified and no cartilage remains in its posterior part. The coronoid is more extensively fused to the dentary.

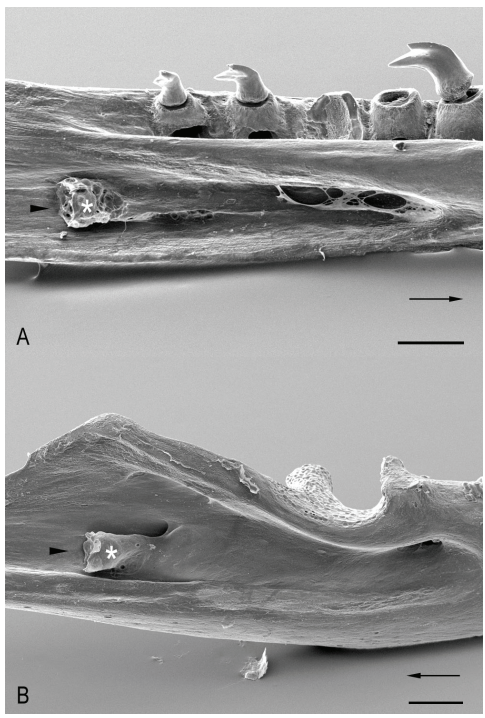

Fig. 4. The meckelian bone in the left ramus of the lower jaw of a juvenile *Hypogeophis rostratus* (Hyro 9); SEM photograph. (**A**) pseudodentary and (**B**) pseudoangular. Meckel's cartilage has been removed and arrowheads indicate its former position. Note the fusion of the ossified part of Meckel's cartilage (meckelian bone, marked with an * in **A** and **B**) to the dermal bones. Arrows point rostrally in both pictures. Scale bar equals 100 µm

In the juveniles, Meckel's cartilage is eroding and is gradually transformed into a meckelian bone that gets incorporated into the pseudodentary and pseudoangular (Fig. 4). The ossification of Meckel's cartilage seems to proceed slowly and gradually from both ends and its length is inversely correlated with that of the postembryonic specimens examined. The retroarticular process is completely ossified. Pedicels of the dentary and coronoid teeth are present and attached to their respective dental laminae.

Branchial and hyoid skeleton

The hyobranchial skeleton is first visible as very faintly stained rods of prechondral condensations and weakly chondrified cartilage at BS 38/39 (Fig. 5A). Ceratohyals and ceratobranchials I to IV are readily identifiable. The ceratohyals are medially connected by a basihyal. The basihyal connects posteriorly to a very faintly indicated basibranchial that connects ceratobranchials I and II medially. One specimen (Hyro 27) of BS 40 shows additional mesenchymatic condensation posterior to ceratobranchial IV (Fig. 5C). It is unclear whether this is an independent entity or part of the ceratobranchial IV anlage. From BS 40/41 on, most elements are well chondrified. A basibranchial is present only as a very faintly stained, thin, thread-like strand of tissue that can be seen in several specimens (Fig 4B,C,D). This thin strand of cell-rich, precartilaginous mesenchyme shows well in histological sections and connects ceratobranchials I, II and III to the basihyal along the midline. It was, however, found to be weakly chondrified in one specimen (Fig. 5C). The embryonic basihyal is somewhat triangular in shape. The ceratohyal and ceratobranchial I are simple, slightly dorsoventrally flattened, cartilaginous rods. Ceratobranchial II is much more strongly compressed. Ceratobranchial III, and particularly ceratobranchial IV are broadened medially but with very slender distal ends that are slightly bend in- and outwards. By BS 45 (Fig. 5E) all elements are well developed. In the sectioned specimens (Hyro 5 and subsequent specimens), the prechondral connection is lost between ceratobranchials I and II, but ceratobranchials II and III are still connected by a small but distinct strand of precartilage. The distal ends of ceratobranchials III are more strongly twisted dorsally and inwards. The distal ends of ceratobranchials IV are also bend dorsally. Ceratobranchial IV is well developed, articulates with ceratobranchial III medially at about one third the length of ceratobranchial III, and is comparatively broad. This appearance does not change much in later stages, although all traces of prechondral connections are gone by BS 46 (in sectioned specimens). BS 46 (Fig. 5F) is very similar to BS 45 (Fig.5 E) in that both have essentially the same shape except that the small, anteromedially directed process

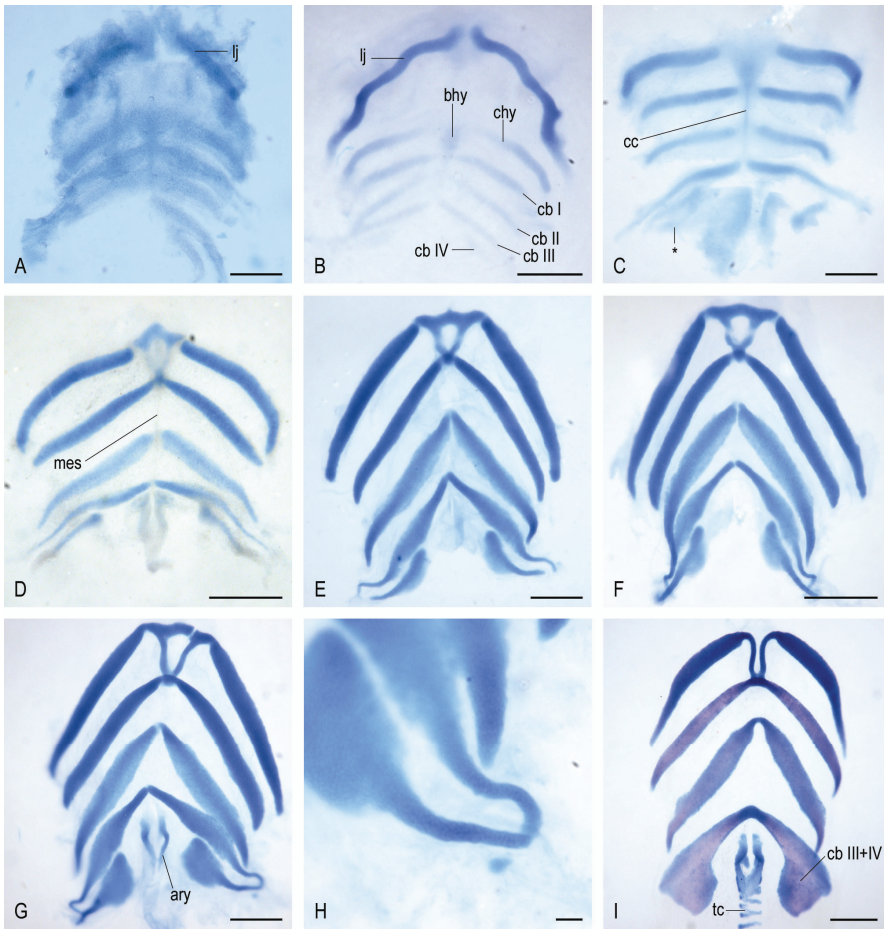

Fig. 5. Development of the hyobranchial skeleton in *Hypogeophis rostratus*, all in ventral view. **A**: BS 38/39 (Hyro 26); note that ceratobranchial III and IV are missing on the right side. **B**: BS 40 (Hyro 25). **C**: BS 40 (Hyro 27); the asterisk (*) marks the mesenchymatic condensation behind ceratobranchial IV. **D**: BS 41/45 (Hyro 31); note the median strand of mesenchyme (mes). **E**: BS 45 (Hyro 11). **F**: BS 46 (Hyro 19). **G**: BS 47 (Hyro 17). **H**: Close-up of **G** showing the cartilaginous nodule posterior of ceratobranchiale IV. **I**: juvenile (Hyro 24); note the fusion of ceratobranchiale III and IV (cb III+IV). ring cartilages posterior of the arytenoid cartilages are tracheal cartilages (tc). **A** and **B** both show the lower jaw (lj) as well. ary, arytenoid cartilage; bhy, basihyale; chy, ceratohyale; cb I, ceratobranchiale I; cb II, ceratobranchiale II; cb III, ceratobranchiale III; cb IV, ceratobranchiale IV; cc, copula communis. Scale bars equal 0.5 mm, except in **H** where the scale bar equals 100 µm.

of the basihyal is absent by BS 46. By BS 47 (Fig. 5G) ceratobranchials II, III, and especially the medial part of ceratobranchial IV, have broadened and are more plate-like. A small, cartilaginous nodule posterior to ceratobranchial IV is present in a single BS 47 specimen (Fig. 5H), but only on the left side. In

juveniles (Fig. 5I), the hyobranchial skeleton has a morphology typical of most adult caeciliid caecilians (Nussbaum, 1977; Wake, 2003), in that the fused ceratohyal and basihyal are M-shaped and fused with the ceratobranchial I while the fused ceratobranchial III+IV is strongly dorsoventrally compressed and greatly expanded.

DISCUSSION

Chondrocranium

Most previous students of caecilian cranial development have remarked on the relatively similar architecture of the chondrochranium among different species (e.g. Peter (1898) and Winslow (1898) for *Ichthyophis glutinosus* and Wake and Hanken (1982) for *Dermophis mexicanus*). All conform to the general pattern of nasal and otic capsules that are chondrified to varying degrees and connected by relatively slender bars of cartilage, the taenia marginalis dorsally and trabecular cartilage ventrally that are interconnected by the pila antotica. *Hypogeophis rostratus* does not deviate greatly from this common pattern, and differs only in some minor aspects from other species. The foramen for the palatine nerve that pierces a conspicuous, broad extension of the parachordal cartilage anterior of the otic capsule, is shared with the Seychellean caeciliid *Grandisonia alternans* (Marcus et al., 1935). Reiss (1996) depicted a similar foramen in a comparable position in the rhinatrematid *Epicrionops petersi*, but considered it to be a carotid foramen. No such foramen is found in the ichthyophid *Ichthyophis glutinosus* (Peter, 1898), the caeciliids *Dermophis mexicanus* (Wake and Hanken, 1982) and *Gegeneophis ramaswamii* (Müller et al., 2005), or the typhlonectid *Typhlonectes compressicauda* (Wake et al., 1985). An unusual characteristic of the chondrocranium of *H. rostratus* is the fusion of the stapes to the parachordal cartilage. This cartilaginous connection persists in juveniles (Fig. 3G). The most variable part of the caecilian chondrocranium seems to be the nasal capsules. Compared to *Ichthyophis* (Ramaswami, 1948; Jurgens, 1971; pers. obs.), the nasal capsule of *H. rostratus* also lacks a dorsal roof but has the floor reduced to a thin, thread-like solum nasi (cornu laterale *sensu* Marcus et al., 1935), with only

the lateral wall being fairly complete. The nasal septum and anterior copula are well developed and the long prenasal process extends beyond the anterior margin of the copula (*contra* Jurgens, 1971). Overall, the extent to which the nasal capsule is developed in *H. rostratus* resembles that of *G. ramaswamii* (Müller et al., 2005) and, to a lesser extent, *D. mexicanus* (Wake and Hanken, 1982). Among "higher caecilians", an informal group comprising the derived families Caeciliidae, Scolecomorphidae and Typhlonectidae (see Nussbaum, 1977, 1979; Duellman and Trueb, 1986), studied so far, *T. compressicauda* has a more extensive nasal capsule but lacks a nasal septum and prenasal process (Wake et al., 1985).

Marcus et al. (1935) described the cranium and Eifertinger (1933) and Marcus (1933) the lower jaw of a "stage 40" embryo of *Grandisonia alternans* based on a reconstruction from serial sections. The extent to which the precartilaginous nasal capsule is developed differs slightly compared to *Hypogeophis rostratus*, although this might be related to differences in differentiating precartilage from other tissue. In my specimens, I furthermore do not see the prechondral condensations above the taenia marginales that Marcus et al. (1935) interpreted as a rudimentary cartilaginous skull roof. More pronounced are the differences in the lower jaw, where Eifertinger (1933) and Marcus (1933) described a large, inward-directed process of Meckel's cartilage just posterior of the symphysis. This process is absent from all specimens of *H. rostratus* examined in this study.

The skull and lower jaw and their constituent bones

The lofty, almost fragile construction of the embryonic chondrocranium is in stark contrast with the heavily ossified skull of adult caecilians. The chondrocranium ossifies almost completely in adult caecilians, with typically only parts of the nasal capsule and anterior nasal septum, and sometimes parts of the orbital and trabecular cartilages, remaining cartilaginous (Wake, 2003). Dermal bones are extensively developed and form a complete skull roof in most adult caecilians (Taylor, 1969b).

In adult caecilians, most of the neurocranium is ossified and forms two large bones, the sphenethmoid and the os basale (Wiedersheim, 1879). These encapsulate the brain and most of the sensory organs and, together with the dermal bones, form the compact caecilian skull. A further characteristic of caecilian skulls is the high degree of fusion that occurs among the neighboring bones. In some cases, as in the os basale, dermal elements fuse with endocranial elements. In this section, I will summarize the composition of the skull and lower jaw of adult of *Hypogeophis rostratus* as proposed by Marcus et al. (1935) and compare their assumptions against my new results. For a comprehensive discussion of bone homologies see Müller et al. (2005).

Parietal. The parietals are large, paired, dermal bones that cover most of the dorsal side of the posterior half of the skull. Marcus et al. (1933, 1935) described the occurrence of a parietal foramen, sometimes closed by a separate interparietal that subsequently fuses with the parietals, and they also described additional postparietal elements. Although not observed in my material, additional smaller bones forming within the suture between larger bones are frequently found in other amphibians and their morphological interpretation is currently debated (e.g., Smirnov, 1997). If present at all, the postparietal is considered to represent a separate centre of ossification of the parietal (Straub, 1985; but see Schoch, 2002).

Frontal. According to Marcus et al. (1935), the frontal in *Hypogeophis* actually represents a temporal because it is derived from the fusion of frontal and prefrontal. They figured a very large prefrontal in a stage 47 embryo (Fig. 6A), which was not observed in similar embryos or any other *H. rostratus* embryo studied herein. According to the observations presented here, the adult frontal of *H. rostratus* arises as a single element and receives no contribution from other ossification centres.

Nasopremaxillary. According to Marcus et al. (1933), the nasopremaxillary of *Hypogeophis rostratus* is composed of the nasal, premaxilla and occasionally the septomaxilla. Subsequent studies have accepted these homologies for caeciliid caecilians in general (e.g., Ramaswami, 1948) although

the septomaxillary was hardly ever observed. None of the *H. rostratus* specimens examined for this study exhibits a clearly distinct septomaxilla. When present in caeciliids, current evidence indicates that its occurrence is rather irregular and transient (Marcus et al., 1935; Wake and Hanken, 1982). Marcus et al. (1935) described most parts of the nasal and premaxilla to be of perichondral, rather than dermal, origin as commonly accepted. Examination of my histological sections shows that the perichondral lamina of the nasal capsule cartilages is unossified by the time nasal and premaxilla are well developed and that the latter two elements are separated from the underlying cartilage, although intimately associated with it. This clearly shows that both nasal and premaxilla do form as dermal bones as in all other tetrapods. Marcus et al. (1935) further suggested a separate origin of the "perichondral" part of the premaxilla and the tooth bearing alveolar process, but from the material examined here it is evident that the premaxilla forms from a single ossification centre. A so-called prepalatine, which Marcus et al. (1935) claimed to be integrated into the oral shelf of the premaxilla is absent.

Vomer. According to Marcus et al. (1935), the vomer is composed of a dermal ossification, the actual vomer, and a replacement ossification at its anterior end, which they homologized with a preethmoid. Such an element is not found in the *Hypogeophis rostratus* specimens examined here, in which the vomer arises as a single, dermal ossification.

Maxillopalatine. The maxillopalatine is a complex bone that incorporates the maxilla, palatine, and lacrimal. The largest contribution to the maxillopalatine in *Hypogeophis* is made by the palatine, with the maxillary forming the outer tooth row and parts of the orbital shelf. The maxilla and palatine each form from single centers of ossification. Marcus et al. (1935) described the palatine to be composed of the palatine and a posterior vomer, which forms the medial part of the choana, but no such separate ossification was observed herein, and the medial wing of the palatine seems to form by simple accretion. Marcus et al. (1935) further described the contribution of a lacrimal to the maxillopalatine. A small, separate ossification is present in the specimens of *Hypogeophis rostratus*

observed herein and corresponds in position to the lacrimal described in *Gegeneophis ramaswamii* (Müller et al., 2005 for detailed discussion).

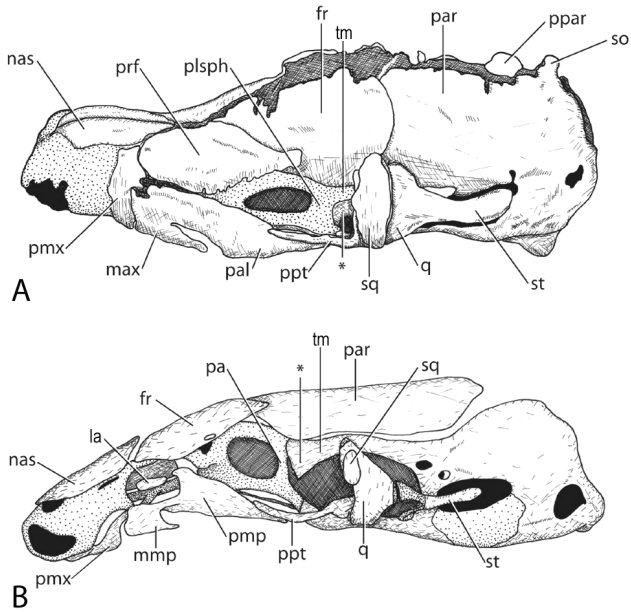

Fig. 6. Skull of embryonic *Hypogeophis rostratus* of stage 47 in lateral view. **A**: redrawn from Marcus et al. (1935). **B**: Results of this study (Hyro 18). Cartilage stippled, bone hatched. fr, frontal; la, lacrimal; max, maxillary; mmp, maxillary part of the maxillopalatine; nas, nasal; pa, pila antotica; pal, palatine; par, parietal; plsph, pleurosphenoid; pmp, palatine part of the maxillopalatine; pmx, premaxillary; ppar, postparietal; ppt, pterygoid process of the palatoquadrate/quadrate; prf, prefrontal; q, quadrate; so, supraoccipital; sq, squamosal; st, stapes; tm, taenia marginalis; asterisk (*) marks the endochondral bone lamina around the taenia marginalis that stretches onto the pila antotica. Labelling in **A** follows Marcus et al. (1935).

Squamosal. The squamosal (paraquadrate *sensu* Marcus et al., 1935) covers most of the cheek region between the eye and the quadrate. It is a dermal ossification that arises lateral to the quadrate and seems to expand rapidly at the time of hatching. Marcus et al. (1935) described the formation of periorbital elements that subsequently fuse to each other and the squamosal. Available specimens of *Hypogeophis rostratus* however did not exhibit any indication of periorbital elements, although I cannot completely rule out their occurrence in embryos very close to hatching, which are missing in my sampling.

Ectopterygoid. First described for caecilians by Wiedersheim (1879), who termed it the postpalatinum, this element was also figured and described by Marcus et al. (1933, 1935) as a free pterygoid bone that is sometimes incorporated into the maxillopalatine. The element they refer to in fact represents the ectopterygoid, as it is formed laterally of the pterygoid process of the pterygoquadrate. The true pterygoid fuses early to the pterygoid process of the quadrate (see below). The ectopterygoid was overlooked by Lawson (1963) and Müller (2003) but correctly identified by Straub (1985). The ectopterygoid is a small bone bordering the posterolateral margin of the maxillopalatine, lateral to the pterygoid process of the pterygoquadrate (Fig. 3G).

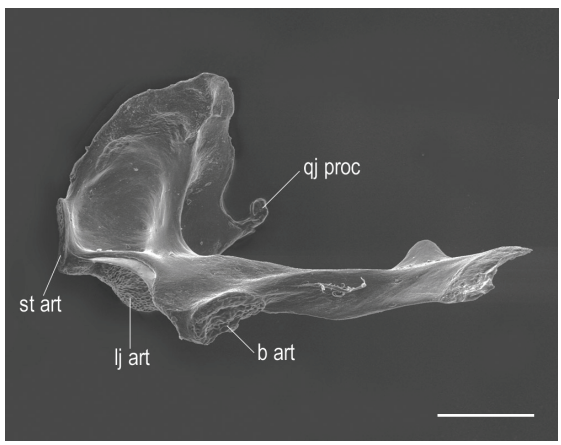

Fig. 7. SEM of medial side of left pterygoquadrate of a juvenile *Hypogeophis rostratus* (Hyro 9) showing the quadratojugular process and the articular facets of the pterygoquadrate. b art, basal articulation; lj art, lower jaw articulation; qj proc, quadratojugular process; st art, stapes articulation. Scale bar equals 250 µm.

Pterygoquadrate. The pterygoquadrate is a complex bone that forms through the fusion of the palatine to the pterygoid process of the quadrate. This fusion has also been reported for *Dermophis mexicanus* (Wake, 2003) and *Gegeneophis ramaswamii* (Müller et al., 2005). The main body of the pterygoquadrate is dorsally connected to the os basale via dense connective tissue. It has three well developed articulatory facets (Fig. 7) for articulation with the stapes (posteriorly), the pseudoangular (ventrally) and the os basale (ventroanteromedially = basipterygoid or basal articulation). The pterygoid portion extends anterior well beyond the pila antotica, overlapping the maxillopalatine dorsally. Anteroventrally a small process is found on the main

body of the pterygoquadrate and assists in supporting the squamosal, which covers most of the pterygoquadrate laterally (Fig. 7). This process was described as a quadrato-maxillary (quadratojugal) by Marcus et al. (1935; see also Peter, 1898 and Reiss, 1996). However, it seemingly forms as membrane bone continuous with the quadrate and homology with a dermal quadratojugal is rejected.

Stapes. Compared to that of other amphibians, the caecilian stapes is a relatively large bone that completely fills the fenestra ovalis in adult specimens. It articulates with the os basale via an anteroventrally directed process and with the posterior side of the quadrate via an anteriorly directed process. It forms as a single element and receives no contributions from the otic capsule, contrary to Marcus et al. (1935) and Marcus (1935). Confirming previous reports (Lawson, 1963; Straub 1985), the stapes of *Hypogeophis rostratus* does not have a foramen for the stapedial artery (*contra* de Beer, 1937).

Os basale. The os basale is by far the largest and most complex bone of the adult skull and comprises the otic capsule, most of the posterolateral neurocranium, and the dermal parasphenoid. According to Marcus et al. (1935) it develops form the following individual elements: basi-, pleuro- (ex-), supra- and infraoccipital, as well as the epiotic, otic capsule, pleuro-, and parasphenoid. Based on my observations, I recognize only the following ossifications as contributing to the adult os basale of *Hypogeophis rostratus*: exoccipital, prootics (likely the otic capsule ossification of Marcus et al., 1935), and parasphenoid. A basioccipital does not occur as a separate ossification. A cartilaginous tectum synoticum is absent and the area above the foramen magnum is covered by membrane bone that extends from the exoccipital and cannot be homologized with a supraoccipital, which is a separate replacement ossification of the cartilaginous tectum synoticum (see also Brand, 1956). Marcus et al. (1935) further considered a short, dorsal process of the otic capsule to represent a rudimentary tectum synoticum, and homologized its ossified successor structure with an epiotic. A separate epiotic ossification is, however, absent. A separate infraoccipital ossification is also absent because the structure in question forms as

membrane bone. A pleurosphenoid ossification is likewise absent (see Müller et al., 2005 for details).

Sphenethmoid. I fully agree with Marcus et al. (1935) that this bone is the most difficult to assess in the caecilian cranium. Marcus et al. (1935) considered the adult sphenethmoid to be composed of unpaired mesethmoid and basiethmoid and paired orbitosphenoid ossifications. The sphenethmoid is the last of the endocranial bones to form. It is first present in late BS 45 embryos, where it consists of a median bony lamina anterior to the pilae preopticae, and extends from the perichondral ossification of the nasal septum. Additionally, continuous lamellae of membrane bone extend laterally and form the anterior wall of the brain cavity (Fig. 3H,I). This membrane bone extends onto the pila preoptica and is in later stages continuous with the perichondral ossification of that structure. In the available specimens, it was not possible to demonstrate more than a single continuous ossification, and it seems possible that this bone, previously considered to be among the most complex products of fusion of multiple ossifications, is formed from only a single (or paired) ossification centre. A final decision requires a more comprehensive ontogenetic series.

Pseudodentary.—The pseudodentary is the tooth-bearing bone of the lower jaw and forms the anterior part and most of the labial side of the jaw from the anterior terminus to the jaw articulation. According to Eifertinger (1933) and Marcus (1933), it is formed by the mentomeckelian, dentary, splenial, coronoid and supraangular. Based on my observations, only the dermal dentary and coronoid (splenial of Eifertinger, 1933 and Marcus, 1933) and the endoskeletal mentomeckelian contribute to form the pseudodentary (Fig. 4).

Pseudoangular.—The remainder of the lower jaw, including the jaw articulation and the retroarticular process, is formed by the pseudoangular, which is, according to Eifertinger (1933) and Marcus (1933) formed by the angular, prearticular, complementary and articular. Of these, only the angular and articular were observed in the material examined here. In BS 40 embryos, a plate-like ossification is present on the lingual side of Meckel's cartilage, anterior of the jaw articulation. It is continuous with the angular via a narrow bony bridge. This

situation resembles Eifertinger's (1933) reconstruction where it was interpreted as a prearticular already fused to the angular. Wake and Hanken (1982) did not find a prearticular in *Dermophis mexicanus* but Müller et al. (2005) reported one in *Gegeneophis ramaswamii*. Meckel's cartilage is transformed gradually into a meckelian bone that is incorporated into the pseudodentary and pseudoangular (Fig. 4).

Hyobranchial skeleton

Knowledge of the hyobranchial skeleton in caecilians stems largely from descriptions of adult morphology (e.g. Wiedersheim, 1879; Brand, 1956; Nussbaum, 1977; Wilkinson and Nussbaum, 1997). The development of the hyobranchial skeleton has been described in *Ichthyophis* spp. (Sarasin and Sarasin, 1887-1890; Peter, 1898; Visser 1963), *Gegeneophis ramaswamii* (Ramaswami, 1948; Müller at al., 2005), *Typhlonectes compressicauda* (Wake et al., 1985), and *Dermophis mexicanus* (Wake, 2003). Wake (1989) further described the metamorphosis of the hyobranchial skeleton in *Epicrionops* spp.

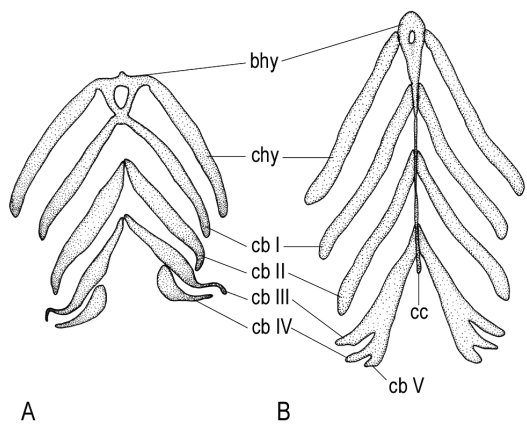

Fig. 8. Hyobranchial skeleton of *Hypogeophis rostratus* at BS 45. **A**: results of this study (Hyro 11). **B**: Redrawn from Gehwolf (1923). bhy, basihyale; chy, ceratohyale; cb I, ceratobranchiale I; cb II, ceratobranchiale II; cb III, ceratobranchiale III; cb IV, ceratobranchiale IV; cb, V ceratobranchiale V; cc, copula communis.

Marcus (1922), summarizing the work of his student S. Gehwolf, and Gehwolf (1923) described the development of the hyobranchial skeleton in *Hypogeophis*. Their descriptions differ from my observations in several points. Marcus (1922) and Gehwolf (1923) described the presence of a fifth ceratobranchial arch that fuses with the fourth and third during ontogeny (Fig. 8B). One of my specimens (Hyro 27, Fig. 5C) shows an undefined prechondral condensation posterior of the fourth arch, although it is unclear if this is a separate entity or an extension of the fourth ceratobranchial. Another specimen (Hyro 17, Fig. 5H) has a tiny cartilaginous nodule on the left side. However, well-developed fifth arch does not seem to be present in general, and Marcus' (1922) and Gehwolf's (1923) descriptions (Fig. 8) are likely based on an aberrant specimen, if correct at all (see critique of Marcus et al. below). Both workers also described a cartilaginous copula communis that connects all arches medially. Such a connection does indeed occur in the form of mesenchyme and precartilage, which may also chondrify to a certain extent (Fig. 5C). A well-developed, cartilaginous copula communis as described for older stages by Marcus (1922) and Gehwolf (1923) is, however, absent in specimens that I examined, although it must be noted that some interspecific variability might be expected here. In general, the observations and interpretations of Marcus (1922) and Gehwolf (1923) differ substantially from those presented here (Fig. 8).

Although the adult morphology of the hyobranchial skeleton of *Hypogeophis rostratus* is similar to that of other caeciliids and typhlonectids, the embryonic morphology as described here shows some obvious differences to *Dermophis mexicanus* (Wake, 2003), *Gegeneophis ramaswamii* (Müller et al., 2005) and *Typhlonectes compressicauda* (Wake et al., 1985). The embryonic basihyal, which usually is shaped like an open V, is triangular with a frontal transverse bar in *H. rostratus*. Ceratobranchial IV is much more prominently developed than in other caeciliids and typhlonectids, in which ceratobranchial IV fuses early in ontogeny to ceratobranchial III and remains vestigial throughout development. In this respect, embryonic *H. rostratus* more closely resemble larvae of *Ichthyophis glutinosus* (Sarasin and Sarasin, 1887–1890; pers. obs.).

Critique of Marcus et al.

The results presented here differ in many instances profoundly from the Marcus et al. (1935) study of skull development in *Hypogeophis* and other studies by Harry Marcus and his students (e.g., Marcus, 1933; Eifertinger, 1933; Marcus et al., 1933; Fig. 6). Marcus et al. (1935) studied only three stages, a BS 40 embryo of *Grandisonia alternans*, a BS 47 embryo of *Hypogeophis rostratus* and a 68 mm long juvenile of *G. alternans*, although it appears that they had more material available, as indicated by reference to a 42 mm "larva" of *H. rostratus* (a species with direct development). In these specimens, the skull of the BS 40 embryo is just beginning to ossify with only small ossifications of vomer and palatine present. The BS 47 embryo, however, shows a high degree of ossification of the skull and the beginning of the formation of the compound bones, such as the maxillopalatine and nasopremaxillary. Given the lack of stages crucial to the analysis if ossification, i.e. stages between BS 40 and 47, it seems unlikely that many of the ossifications reported by Marcus et al. (1935) could have actually been observed.

One of the difficulties in evaluating the results of Marcus and his co-workers is their liberal mingling of two different, though closely related, species. For their studies, Marcus and co-workers used specimens of *Hypogeophis rostratus* and *H. alternans*, of which the latter is now referred to the genus *Grandisonia* (Taylor, 1968). Although such a procedure might not be so problematic under certain circumstances, Marcus viewed both species as interchangeable, as is made clear by his statement: "…in this study both species will not be separated but the most instructive specimen will be chosen, unconcerned of the species." (Marcus, 1908:696 [author's translation]) and specimens were thus often simply referred to as *Hypogeophis*, without indicating which species was actually examined (e.g. Marcus, 1909). It appears that the two species do indeed have a very similar early embryogenesis (Brauer, 1897, 1899), but differences in later ontogeny are obvious, and advanced embryos of *H. rostratus* are easily distinguished from those of *G. alternans*. *Grandisonia*, which today comprises an additional 3 species (Nussbaum and Wilkinson, 1989;

Wilkinson and Nussbaum, 2005; but see Straub, 1985), furthermore seems to differ in life history from *H. rostratus*. At least some species of *Grandisonia* possess larval characters (e.g., neuromasts, gill slits) upon hatching (Parker, 1958; pers. obs.), which are lacking in the direct-developing *H. rostratus*. Some of the differences between the observations presented here and those of Marcus and co-workers might therefore be the result of specific differences, although it seems unlikely that *G. alternans* or indeed any *Grandisonia* spp. shows the characteristics described by Marcus et al. (1935), given that these are neither present in *H. rostratus* nor in the closely related *G. ramaswamii* (Müller et al., 2005). Straub (1985) called into doubt the identity of some the material identified as *G. alternans* by Marcus et al. (1933, 1935) and tentatively treated all specimens used by Marcus and co workers as *H. rostratus*.

In some instances, Marcus and co-workers explicitly mention studying *Hypogeophis rostratus*. Here, other explanations must be sought for the reported differences. A substantial proportion appear to stem from the different methods used to observe and document developmental morphology, paired with over-interpretation of the evidence. Marcus and co-workers based their observations on reconstructions from serial sections. Although this method can produce greatly magnified and very instructive models, it sometimes fails to reproduce the correct morphology (compare head curvature in Fig. 6 and hyobranchial skeleton shape in Fig. 8), because serial sections can be difficult to align and interpret. Separate elements, such as bones that are in close proximity, can be mistakenly interpreted as a single element, whereas a single but complicated structure might appear to be formed by several individual elements. For instance, Marcus et al. (1935) described the palatoquadrate to be continuous with the stapes. From my observations, it is clear that both elements are never continuous with each other, but at times they tightly abut each other, with the border between them being easily overlooked in sectioned specimens. In another instance, Eifertinger (1933) and Marcus (1933) interpreted the dorsal tip of the angular to represent a so-called complementare because it appears somewhat irregular and can sometimes be found to form separately. A more probable explanation in line with my

observations is that Eifertinger (1933) wrongly interpreted a mere process of the angular as an independent element. This is supported by Eifertinger's (1933) remark that it could only be seen in single sections. Eifertinger (1933) sectioned his material at 24 µm, which is rather thick and can therefore easily obscure continuity of an element. Where mentioned, most of the specimens used by Marcus and co-workers were also sectioned quite thickly (up to 30 µm), which is even more relevant in the very small, embryonic skulls considered here, where the anlagen of individual bones are sometimes just a few micrometers apart.

Most cases of disagreement between this and previous studies stem from what seems to be over-interpretation of the observed morphology by Marcus and co-workers that, together with the very limited number of embryonic stages studied, led to several errors. It is evident that weakly ossified areas, narrowing and broadening of an element, or indentations within an element were in many cases interpreted as evidence for a previous fusion between two independent elements, even though those postulated elements were never observed as independent structures prior to their presumed fusion. Reports of a quadratojugal (quadrato-maxillary *sensu* Marcus et al., 1935; Fig. 7) and a posterior vomer (Marcus et al., 1935) are such cases. In other instances, the presence of individual bones during ontogeny was assumed if bone was found in the skull in a position that corresponds to a separate ossification in the skull of Palaeozoic forms. The description by Marcus et al. of a pleurosphenoid and basi- and supraoccipital seem to be based on such an assumption. As can be seen in Figure 6A, Marcus et al. (1935) correctly figured the bone that envelops the pila antotica as a lamella of membrane bone that extends from the taenia marginalis onto the pila. In their description, however, they interpreted this bone to be a pleurosphenoid although no separate ossification occurs in the pila antotica. The description of these elements without their actual observation was greatly facilitated by Marcus' assumption that caecilians are direct descendants of Palaeozoic forms (so-called stegocephalians), specifically aistopods (Marcus et al., 1933; Marcus, 1933) and unrelated to other living amphibians. He was followed in that by all of his students, which resulted in a sometimes bizarre distortion of the evidence.

Eifertinger (1933), for example, in the concluding remarks to his study of the caecilian lower jaw, stated that the observed high number of individual elements supports a grouping with aistopods, yet acknowledges that lower jaws of aistopods were unknown at that time.

stage	skull	lower jaw
38/39		dentary
40/41	palatine vomer	angular mentomeckelian
	exoccipital premaxilla prootics pterygoid	
41/45	quadrate	articular
	frontal maxilla parietal pterygoquadrate	
45	anterior part of parasphenoid squamosal	coronoid
	lacrimal maxillopalatine nasal sphenethmoid stapes	pseudoangular pseudodentary
46	posterior part of parasphenoid	
47	ectopterygoid lacrimal-maxillopalatine	
	nasopremaxillary	
>47	os basale	

Table 1. Ossification sequence of *Hypogeophis rostratus*. Elements are listed according to their first appearance. The sequence of the elements within boxes could not be resolved.

Ossification sequence (Table 1)

Some of the available earlier embryos are decalcified to various degrees, likely as a result of fixation or storage, so I have relied mostly on the sectioned material and later, better-preserved embryos for reconstructing the ossification sequence of *Hypogeophis rostratus*. The dentary is the first ossification to appear, followed by the angular, vomer, palatine, mentomeckelian, exoccipital, pterygoid and premaxilla. These are followed by the prootics. Shortly after ossification of the articular and quadrate, the parietal, frontal, and maxilla appear and the pterygoid fuses with the quadrate to form the pterygoquadrate. This is followed by ossification of the coronoid, parasphenoid, squamosal, and later by the nasal, lacrimal, sphenethmoid and stapes, and the formation of the maxillopalatine, pseudoangular and pseudodentary. The last dermal elements to appear are the ectopterygoid and the posterior part of the parasphenoid, at which point the lacrimal fuses to the maxillopalatine and, at a later stage, the nasopremaxillary and os basale form.

The ossification sequence reported here for *Hypogeophis rostratus* is relatively similar to that of *Gegeneophis ramaswamii* (Müller et al., 2005). Differences include the early formation of the squamosal as compared to the nasal. Information on ossification sequences of caecilians is very fragmentary and comprehensive ossification sequences are only available for *Dermophis mexicanus* (Wake and Hanken, 1982) and *G. ramaswamii* (Müller et al., 2005). The direct developing *H. rostratus* and *G. ramaswamii* have a more similar ossification sequence as compared to that of the viviparous *Dermophis mexicanus.* All three species are characterized by derived reproductive modes and a functional correlation between these and the observed ossification sequences might be expected. In *D. mexicanus*, for example, the comparatively early onset of the ossification of the jaw articulation is seemingly correlated with intrauterine feeding at an early stage (Wake and Hanken, 1982). Müller et al. (2005) reviewed the literature with regards to ossification sequences in caecilians, and concluded that the available data is currently insufficient to enable a robust inference of the evolution of ossification sequences in caecilians.

Conclusion

From the mid 1900s until his emigration from Germany in 1938, fleeing persecution by the Nazi regime (Tonutti, 1977), Harry Marcus and his students conducted a very extensive research program on the morphology of just two caecilian species, *Hypogeophis rostratus* and *Gegeneophis alternans*. They contributed a sizeable amount of data that forms a considerable proportion of our still relatively scant knowledge of caecilian morphology. Their work, due to the paucity of data on other species, was often seen as representative for all caecilians and thus has been very influential in other workers' interpretations of caecilian morphology (e.g., Ramaswami, 1948; Visser, 1963; Carroll and Currie, 1975), and still resonates in the debate about the phylogenetic position of caecilians. Subsequent workers, however, have pointed out inconsistencies (Brand, 1956) and, more recently, incongruence (Wake and Hanken, 1982; Müller et al., 2005) with results reported by Marcus and students. Here I have shown that many of the reported ossification centers supposedly forming the adult cranium are absent during development, and that their description was based largely on a misinterpretation of the observed morphology promoted by misplaced phylogenetic assumption. In my work, I have focussed on works of Marcus and his students that concern the morphology and development of the skull, lower jaw and hyobranchial skeleton (Marcus, 1922, 1933, 1935; Gehwolf, 1923; Marcus et al. 1933, 1935; Eifertinger, 1933). Marcus and students also worked on other organ systems in caecilians (e.g., Marcus, 1923; Marcus and Albrecht, 1936) and although no attempt has been made to assess the accuracy of those observations, I suggest that these publications should be approached carefully and caution against their uncritical use. Problems with previous works and the small amount of available comparative data should encourage new, sorely needed investigations into caecilian developmental biology.

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Appendix. List of material examined.

stage	study ID (collection number)	total length in mm	preparation
37	Hyro 30 ex. ZMB 35003-016	19	cleared and stained
38	Hyro 12 ex. ZMB 34665-760	24	cleared and stained
38/39	Hyro 13 ex. ZMB 34665-760	24	cleared and stained
38/39	Hyro 26 ex. ZMB 34665-760	25	cleared and stained
40	Hyro 1ex. ZMB 35044-070	26	serial sections
40	Hyro 27 ex. ZMB 34665-760	24	cleared and stained
40	Hyro 25 ex. ZMB 34665-760	25	cleared and stained
40/41	Hyro 15ex. ZMB 35003-016	27	cleared and stained
41	Hyro 16ex. ZMB35003-016	28	cleared and stained
41	Hyro 28 ex. ZMB 34665-760	27	cleared and stained
41	Hyro 14ex. ZMB 34665-760	28	cleared and stained
41/45	Hyro 29 ex. ZMB 34665-760	28	cleared and stained
41/45	Hyro 4ex. ZMB 35044-070	36	serial sections
41/45	Hyro 31ex. ZMB 35003-016	30	cleared and stained
45	Hyro 32ex. ZMB 35003-016	30	cleared and stained
45	Hyro 5ex. ZMB 35044-070	38	serial sections
45	Hyro 11ex. ZMB 34865-887	42	cleared and stained
46	Hyro 2ex. ZMB 35044-070	50	serial sections
46	Hyro 19ex. ZMB 34665-760	47	cleared and stained
46	Hyro 20ex. ZMB 34665-760	45	cleared and stained
46+	Hyro 6ex. ZMB 35044-070	50.7	serial sections
47	Hyro 21ex. ZMB 34665-760	45	cleared and stained
47	Hyro 18 ex. ZMB 34665-760	45	cleared and stained
47	Hyro 17ex. ZMB 34665-760	51	cleared and stained
47	Hyro 7ex. ZMB 35044-070	56	serial sections
juvenile	Hyro 22ex. ZMB 34761-864	73	cleared and stained
juvenile	Hyro 8ex. ZMB 34761-864	73	serial sections
juvenile	Hyro 23ex. ZMB 34761-864	82	cleared and stained

juvenile	Hyro 24ex. ZMB 34761-864	82	cleared and stained
juvenile	Hyro 9 ex. ZMB 34761-864	86	cleared and stained; SEM
juvenile	Hyro 10ex. ZMB 34761-864	127	cleared and stained
adult	Hyro 33 ex. ZMB 34565-578	240	manual dissection

CHAPTER 3

Morphology of larval caecilian amphibians (Amphibia: Gymnophiona): implications for caecilian evolution

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ABSTRACT Caecilians are snake-like amphibians that generally possess a heavily ossified skull associated with the burrowing life-style found in most species of the group. Among caecilians, two types of general skull morphology are found. The majority of species possess stegokrotaphic skulls in which the temporal region is completely covered by bone, while a smaller number of species are characterized by zygokrotaphic skulls in which a temporal window is present between the squamosal and the frontal and parietal. Most authors considered stegokrotaphy to have secondarily evolved in caecilians as an adaptation to their burrowing life-style, while some authors have argued for primary stegokrotaphy in caecilians. The latter view has recently received some support through the discovery of the putative stem-line caecilian *Eocaecilia micropodia*, which possesses a stegokrotaphic skull. To reconstruct the pattern of the skull in the last common ancestor of living caecilians, we studied the skull and associated musculature in larvae and adults of representatives of all lineages known to have free-living larvae. Based on the data presented here, we conclude that the ancestral caecilian likely possessed a zygokrotaphic skull in which the primary adductor musculature extended onto the dorsal side of the skull as in rhinatrematids caecilians. Our account provides the first descriptions of the skull and hyobranchial skeleton of larval *Uraeotyphlus* spp., larval and adult *Praslinia cooperi*, larval *Sylvacaecilia grandisonae* and larval *Grandisonia* spp., as well as of the musculature of larval *Epicrionops* spp. and *Rhinatrema bivittatum*, larval *Uraeotyphlus* spp., larval and adult *P. cooperi*, larval *S. grandisonae* and larval *Grandisonia spp.*

INTRODUCTION

The caecilians or Gymnophiona form the smallest of the three groups of living amphibians. Caecilians are elongate, snake-like amphibians completely lacking limbs and girdles, which readily distinguishes them from frogs and salamanders, and occur primarily in the wet and seasonal tropics of South and Middle America, Africa, and Asia (Wilkinson & Nussbaum, 2006). The majority of species are surface-cryptic or burrowing as adults, except for the South American

Typhlonectidae, which are semiaquatic or aquatic as adults (Taylor, 1968). Rhinatrematid, ichthyophiid, uraeotyphlid and some caeciliid caecilians further have a free-living larva that has an aquatic or semiaquatic lifestyle until metamorphosis (Taylor, 1968; Largen *et al*., 1972; Wilkinson, 1992a; Himstedt, 1996,). One of the most conspicuous features of caecilians is the heavily ossified skull, which seems to be an adaptation to the burrowing life-style seen in most species.

Tetrapod skulls can be classified into three different groups – gymnokrotaphic, zygokrotaphic, and stegokrotaphic – relating to the degree of coverage of the temporal fossae by bone. The vast majority of living tetrapods is characterized by gymnokrotaphic or zygokrotaphic skulls (see contributions in Hanken & Hall, 1993). While zygokrotaphic skulls have a partially covered temporal fossa, gymnokrotaphic skulls, which are found in most urodeles and anurans, are characterized by a completely open temporal fossa and the jaw adductor musculature is fully exposed (Duellman & Trueb, 1986). In stegokrotaphic skulls, the adductor musculature is fully contained inside the adductor chamber and the temporal region is completely covered by dermal roofing bones.

Most adult caecilian amphibians have stegokrotaphic skulls but the basal-most lineage, the Rhinatrematidae, is characterized by having a zygokrotaphic skull. Zygokrotaphic or weakly zygokrotaphic skulls are also found in all other major groups of caecilians, the Ichthyophidae, Scolecomorphidae, Caecilidae and Typhlonectidae (Taylor, 1969). In all zygokrotaphic forms, with the exception of the Rhinatrematidae, the adductor mandibulae musculature is contained inside the adductor chamber and does not extend onto the outside of the skull. In Rhinatrematids however, the adductor mandibulae leaves the adductor chamber through the gap between squamosal and parietal and inserts broadly onto the dorsal side of the skull, as seen in many urodeles and anurans (Nussbaum, 1983).

While most previous workers have regarded stegokrotaphy in caecilians to be derived from gymnokrotaphic ancestors (e.g. de Beer 1931, Parsons &

Williams 1963), a few have questioned this and assumed it to be plesiomorphic (e.g. Kingsley, 1902; Marcus *et al.*, 1935). In the light of a new, palaeontologically informed hypothesis on caecilian ancestry (Carroll & Currie, 1975), Nussbaum (1983) reviewed the arguments for and against primary stegokrotaphy in caecilians. He compared head morphology across the group and drew attention to the unique secondary mechanism of jaw closure in caecilians. In light of his observations, he concluded that all available evidence points towards a secondary acquisition of stegokrotaphy in caecilians from gymnokrotaphic ancestors.

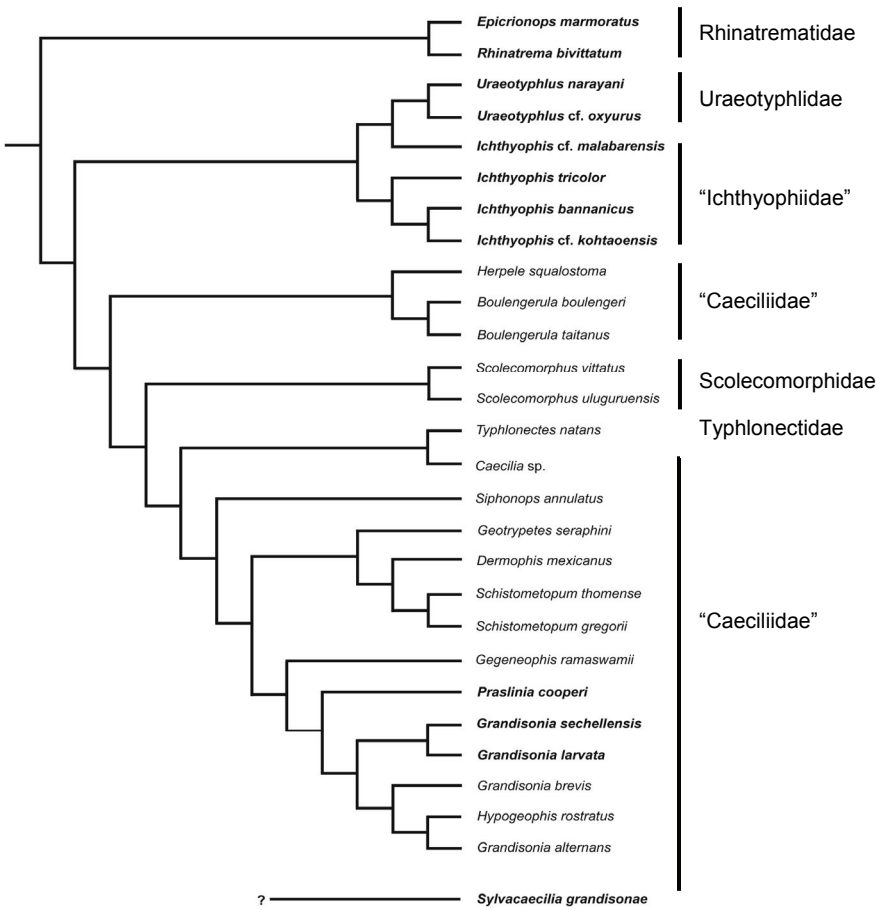

Fig. 1. Phylogenetic relationships of caecilians, from Wilkinson et al. (2003) with additional taxa from Gower et al. (2002). All taxa known to possess free-living larvae are in boldface.

In 1993, Jenkins and Walsh described *Eocaecilia micropodia*, a putative stem-line gymnophionan that is characterized by a stegokrotaphic skull, and they argued for stegokrotaphy to be the plesiomorphic condition for caecilians, with zygokrotaphy in rhinatrematids being derived. Carroll (2000) provided an updated reconstruction of *E. micropodia* and also considered stegokrotaphy to be plesiomorphic for caecilians. He argued that ichthyophiid caecilians are more likely the most "primitive" forms within the group and considered the zygokrotaphic condition in rhinatrematids, where the adductor muscles leave the adductor chamber to be uniquely derived. A similar conclusion was drawn by Jenkins *et al.* (in press) from their comprehensive study of the anatomy of *E. micropodia*.

So far, adult caecilian morphology has been primarily examined with regard to the stegokrotaphy *vs.* zygokrotaphy debate and proposed evolutionary and adaptive scenarios. Although many caecilians are characterized by derived reproductive modes such as direct development and viviparity (Wilkinson & Nussbaum, 1998; Wake, 2006), several taxa including the rhinatrematids, ichthyophiids, uraeotyphlids as well as some caeciliids have a larval stage in their life history that metamorphoses into the adult (Fig. 1). Metamorphosis in caecilians is poorly characterized (Wake, 2006) and larval morphology has so far only received scant consideration. Reiss (1996) described the morphology of the palate of the skull of larval *Epicrionops* and Sarasin & Sarasin (1887-1890), Ramaswami (1947) and Visser (1963), among others, described the morphology of the skull of larval *Ichthyophis*. Wake (2003) provided short descriptions and figures of the larval skull of *Epicrionops bicolor* and *Ichthyophis sp.*. Uraeotyphlids were long considered to be direct developing until Wilkinson (1992a) described the larva of *Uraeotyphlus oxyurus*, but no data are available on its skull morphology or myology. As in uraeotyphlids, information on the morphology of caeciliid larvae is restricted to external morphology (Parker, 1958; Largen et al., 1972). Even less information is available on the musculature of larval caecilians. Edgeworth (1935) commented on the musculature of larval *Ichthyophis* in his study of vertebrate cranial musculature, and Haas (2001)

described the primary jaw adductor musculature of larval *I. kohtaoensis*. The only detailed study of larval musculature in caecilians to date is the description of the cranial musculature of larval *I. kohtaoensis* (Kleinteich and Haas, 2007).

Here we provide accounts of the head morphology of larval and adult caecilians representing all lineages that still possess a free-living larva, and discuss the implications of larval morphology for the reconstruction of caecilian evolution.

MATERIAL AND METHODS

We examined larval and adult specimens of *Epicrionops lativittatus*, *Rhinatrema bivittatum*, *Ichthyophis bannanicu*s, *I.* cf. *kohtaoensis*, *Uraeotyphlus* cf. *narayani*, *U. oxyurus*, *Praslinia cooperi*, *Sylvacaecilia grandisonae*, *Grandisonia sechellensis* and larval *G.* cf. *larvata* (see Appendix 1 for further details). We have tried to investigate conspecific larvae and adults wherever possible, although in some cases only one of these stages of a particular species was available. Our study focuses primarily on the morphology of the larval and adult skulls and hyobranchial skeletons and the musculature associated with the mandibular arch and ceratohyal arch.

Most specimens were manually dissected and subsequently cleared and stained. A few specimens were available as serial sections. Specimens selected for dissection were double stained for bone and cartilage using a slightly modified protocol based on Taylor & Van Dyke (1985). Dissections were performed after application of the Alcian blue stain (Haas, 2001). Where necessary, contrast of the specimens was further enhanced using the method of Bock & Shear (1972). Dissections were performed under a Nikon SMZ-U stereomicroscope equipped with a camera lucida.

Skulls of adults and larvae of some of the taxa investigated here, or closely related species, have previously been described (e.g., Sarasin & Sarasin, 1887-1890; Ramaswami, 1941; Taylor, 1969; Nussbaum, 1977, 1979; Straub, 1985; Wake, 1987, 2003; Reiss, 1996) and their skull morphology is described

here in sufficient enough detail only to enable a comparison with previously undescribed taxa. Muscle terminology follows Kleinteich & Haas (2007).

DESCRIPTIVE ACCOUNT

Skull

Epicrionops lativittatus

The larval skull is comparatively slender (Fig. 2). The anterior-most part of the skull is formed by the cartilaginous nasal capsule, which is not covered by bone anterolaterally and anterodorsally. A conspicuous feature is the wing-like anterior copula that forms the anterior boundary of the naris. The posterolateral border of the naris is formed by a small septomaxilla. Paired nasal, frontal and parietal bones dominate the dorsal aspect of the skull. All bones are in close proximity along the dorsal midline, with only the frontal partly abutting its antimere, thus leaving the sphenethmoid narrowly exposed between the nasals and frontals and the brain cavity between the parietals. The nasal has a pronounced, relatively slender anteromedial process and a broader anterolateral process, which leave the anterior half of the dorsal fenestra of the nasal capsule exposed between them. The frontal is a relatively simple plate-like bone, slightly larger than the nasal. The parietal is more than twice as large as the frontal and almost rectangular in dorsal view. It has a distinct posterolateral process that articulates with the dorsal process of the squamosal. The dorsal border of the foramen magnum is formed by a slender bony bridge that spans between the ossified otic capsules.

The paired premaxilla consists of a well-developed dental lamina that bears bicusped teeth, a relatively narrow palatal shelf between the dental lamina and the vomer, and a long, slender dorsomedial alary process that extends dorsally between the anteromedial processes of the nasals. Dorsal to the premaxilla, at the posterior margin of the naris, is a small septomaxilla. Both are followed by the maxilla, which covers most of the dorsolateral, lateral and ventrolateral side of the nasal capsule.

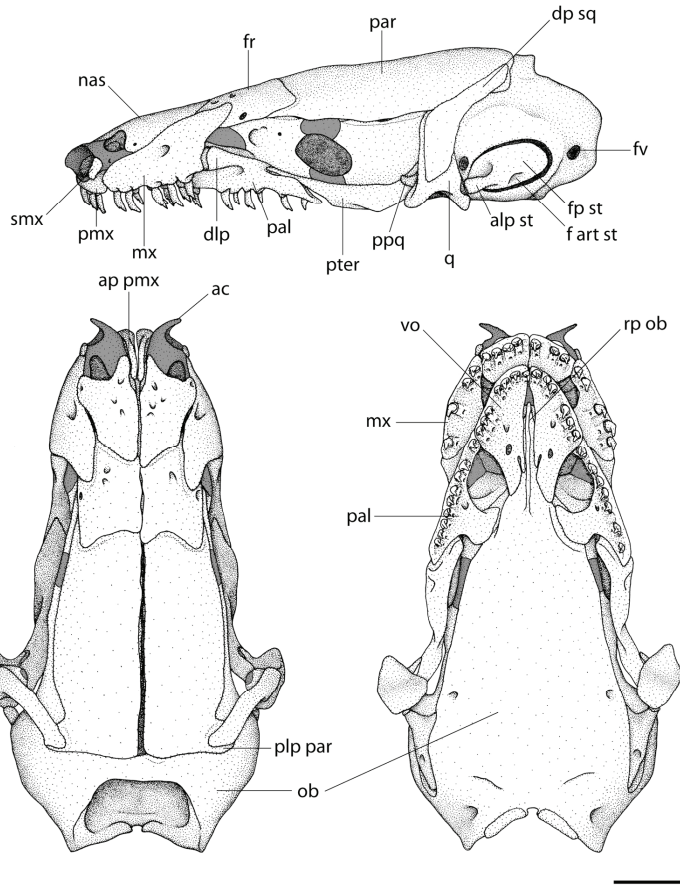

Fig. 2. Larval skull of *Epicrionops lativittatus* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bone is stippled and cartilage stippled with grey shading. ac, anterior copula; alp st, anterolateral process of stapes; al pmx, alary process of premaxilla; dlp, dorsolateral process of palatine; dp sq, dorsal process of squamosal; f art st, stapedial artery foramen; fp st, footplate of stapes; fr, frontal; fv, vagus foramen; mx, maxilla; nas, nasal; ob, os basale; pal, palatine; par, parietal; plp par, posterolateral process of parietal; pmx, premaxilla; ppq, pterygoid process of quadrate; pter, pterygoid; q, quadrate; rp ob, rostral process of os basale; smx, septomaxilla; vo, vomer. Scale bar equals 1 mm.

A broad orbital process of the maxilla extends dorsally, abuts the lateral edge of the nasal and overlaps the anterolateral part of the frontal. This orbital process also forms the anterior border of an otherwise ill-defined orbit. The maxilla bears bicusped teeth. Ventrally, posterior of the premaxilla and medial to the maxilla is the vomer, a paired, somewhat triangular bone that bears the vomerine tooth row at its anterolateral margin and forms the anteromedial border

of the choana. Medially, the vomers are separated by the rostral process of the parasphenoid part of the os basale, which the vomers overlap ventrally along their medial margins. Posterior of vomer and maxilla is the palatine, which is separate of the maxilla. The anterior tooth bearing part of the palatine forms the lateral border of the choana while the posterior choanal border is formed by the medial process of the palatine. This medial process abuts the sphenethmoid medially and also partly overlaps with the parasphenoid part of the os basale ventrally. At its lateral side, ventral to the position of the eye, the palatine has a dorsolateral, anteriorly directed process that abuts the posterior edge of the maxilla. The palatine teeth form the posterior continuation of the vomerine tooth row. Both vomer and palatine bear bicusped teeth. The posterior end of the palatine is broadly overlapped by the pterygoid dorsally, which fits a shallow fossa of the palatine. The pterygoid forms a long, slender bone that extends in parallel along the ventrolateral edge of the os basal. A large dorsal process of the pterygoid invests the anteromedial side of the quadrate. This connection with the quadrate is intimate and it covers most of its medial side, reaching almost to the dorsal tip of the quadrate. The pterygoid further shows some torsion along its long axis. Apart from the dorsal process, the pterygoid is oriented parallel to the main longitudinal axis of the skull and thus in line with the palatine tooth row when seen in lateral view.

The quadrate forms an almost vertically oriented, bluntly rounded cone-shaped bone. It has a short pterygoid process that fits a groove on the dorsolateral side of the pterygoid. Most of the lateral aspect of the quadrate is covered by the squamosal, to which it is syndesmotically bound. The ventral side of the quadrate forms the articulation for the lower jaw and bears an anterior and posterior process separated by a relatively deep medial groove. The dorsal-most tip of the quadrate is cartilaginous. At the posteroventral edge of the quadrate is a depression that articulates with the anterolateral process of the stapes. The footplate of the stapes fits the fenestra ovalis almost completely. The stapes has a relatively long and slender anterolateral process that articulates with the quadrate anteriorly and an inconspicuous anteromedial process that articulates with the os

basale at the anteroventral end of the fenestra ovalis. The base of the anterolateral process is pierced by the passageway for the stapedial artery. Most of the endocranium is ossified, apart from the orbital and trabecular cartilage remnants between the os basale and the sphenethmoid and parts of the nasal capsule, including the lamina orbitonasalis. The parasphenoid is already fused to the posterior endocranium to form the os basale and the entire lateral side of the brain cavity formed by the os basale is closed, apart form various nerve and vessel formina.

The lower jaw of the larvae has a slightly pointed, parabolic shape in ventral view, like almost all caecilians. In lateral view, the lower jaw is straight and the retroarticular process is level with the rest of the lower jaw. The length of the prearticular process is a little less than one third of the total length of the lower jaw. A coronoid tooth row (see Müller et al., 2005) is present lingually of the dentary tooth row and has a length of about three fifths of the latter.

In the adult, most of the nasal capsule is covered by bone apart from the cartilaginous anterior copula that surrounds the nares (Fig. 3). The characteristic anterolateral wing has disappeared and the anterior copula forms a simple, dome-shaped structure with a comparatively much smaller naris. The nasal has expanded anteriorly, reaching the tip of the snout, and covers almost the entire dorsal aspect of the nasal capsule. An anteromedial and anterolateral process is no longer distinct, but a peculiar anteromedial window is present between the nasals through which the alary processes of the premaxillae are visible. Frontal and parietal are similar in shape to those in larval *Epicrionops* but show some tighter suturing with neighbouring elements.

The septomaxilla is slightly larger than in the larvae but still by far the smallest skull bone. It no longer forms part of the external naris but lies laterally to the anterior copula, some distance behind the naris, in a gap bordered by nasal, maxillopalatine and premaxilla. The premaxilla is similar in shape to those of larvae. The maxilla is fused with the palatine into the maxillopalatine. The orbital shelf of the maxillary part has greatly expanded posteriorly and dominates the lateral aspect of the skull. In the centre of the orbital shelf is the orbit, which is

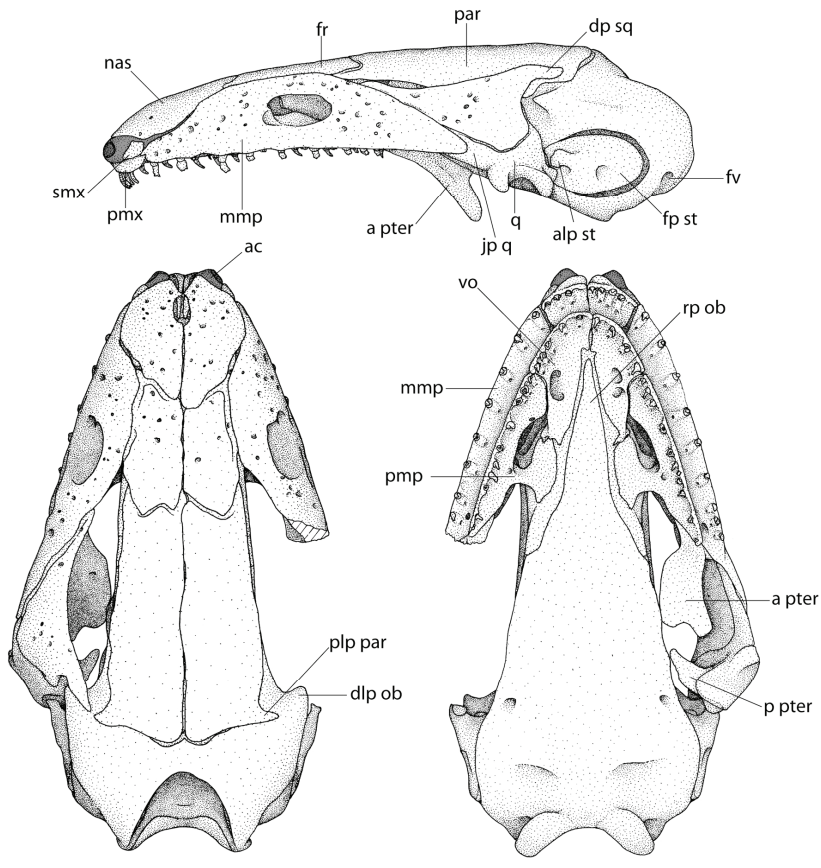

Fig. 3. Adult skull of *Epicrionops lativittatus* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bone is stippled and cartilage stippled with grey shading. a ppter, anterior pterygoid; ac, anterior copula; alp st, anterolateral process of stapes; dlp ob, dorsolateral process of os basale; dp sq, dorsal process of squamosal; fp st, footplate of stapes; fr, frontal; fv, vagus foramen; jp q, quadratojugal process of quadrate; mmp, maxillary part of maxillopalatine; nas, nasal;p pter, posterior pterygoid; pmp, palatine part of maxillopalatine; par, parietal; plp par, posterolateral process of parietal; pmx, premaxilla; q, quadrate; rp ob, rostral process of os basale; smx, septomaxilla; vo, vomer. Scale bar equals 1 mm.

bordered completely by the maxillopalatine. The orbital shelf is sutured to the nasal and overlaps the entire lateral-most edge of the frontal, where it reaches its highest point. Posterior to its articulation with the frontal, the orbital shelf tapers ventrally and has a broad, oblique suture with the squamosal. At its posterior-most end, the orbital shelf overlaps the quadratojugal process of the quadrate

laterally. This articulation of the maxillopalatine with the quadrate is mediated by a slight, posterolateral extension of the orbital shelf. The dental and palatal shelves of the maxillary part of the maxillopalatine do not contribute to this posterolateral extension and the maxillary tooth row ends before said extension begins. The vomer is also similar in shape to those of larvae. The medial edges of the vomers however, are not parallel as in larvae but diverge posteriorly, leaving a broader medial gap between them. The vomer has further no longer an overlap or contact with the rostral process of the os basale. Palatine and maxilla are fused into the maxillopalatine. Ventrally, the palatine part of the maxillopalatine is similar in shape to the palatine of the larva, except for a moderately broadened palatal shelf that forms a broader suture with the vomer and an extended medial part of the medial process that contacts the sphenethmoid. As a result, the choana is more elongated and slit-like in shape compared to that of the larva. The single pterygoid of the larva is divided into an anterior and posterior part in the adult (see Reiss, 1996). The anterior part is broad, with a pronounced pterygoid flange that forms the site of origin of the *m. pterygoideus*. The anterior pterygoid is also more obliquely oriented that the larval pterygoid, and forms an angle of almost 45° to the palatine tooth row. The posterior part of the pterygoid is much smaller than the anterior and has two main processes. A dorsal process invests part of the medial side of the quadrate, as in the larva, and a medial process braces the quadrate against the os basale horizontally. The anterior and posterior part of the pterygoid are both syndesmotically bound to the os basale.

The quadrate has a large, anteriorly directed quadratojugal process that articulates with the squamosal dorsally and the maxillopalatine anteriorly. The main body of the quadrate is slightly broader than in the larva and has a more oblique orientation. The pterygoid process is very inconspicous. The lateral aspect of the quadrate is still largely covered by the squamosal. The squamosal, however, has undergone a considerable shape change. The dorsal process of the squamosal is slightly shorter than in the larva but more massive and rounded in cross section. It no longer articulates with the posterolateral process of the parietal but is instead bound by a strong tendon to the dorsal process of the os

basale. The main body of the squamosal, lateral to the quadrate, has expanded anteriorly, almost reaching the level of the suture between the frontal and the parietal. The stapes is similar in general shape to that of the larva, but has a slightly higher footplate and a proportionately shorter and stouter anterolateral process, which articulates with a fossa on the posterior side of the quadrate. The os basale is proportionately more slender and elongated than that of the larva, but otherwise of similar general shape. A pronounced difference, however, is the presence of a broad, conical process on the anterodorsal edge of the otic capsule. This process fits a posterior notch of the squamosal and articulates with the dorsal process of the squamosal via a very strong tendon.

The shape of the lower jaw is comparable to that of the larva. The retroarticular process has a similar length and orientation as in the larva but is more massively developed. The coronoid tooth has expanded to about four fifths of the length of the dentary tooth row.

Ichthyophis bannanicus* and *I.* cf. *kohtaoensis

Both species of *Ichthyophis* have a very similar skull morphology in larvae and adults and the following description applies to both species. The overall habit of the larval skull (Fig. 4) is similar to that of larval *Epicrionops lativittatus*, even though the larval *Ichthyophis* skull is broader and of a more robust appearance. This is mainly attributable to a broader rostral region, which is as broad as the otic capsule. The anterior nasal capsule is very similar to larval *E. lativittatus* and the anterior copulae are also characterized by anterolateral, wing-like extensions, albeit slightly smaller. The posterior border of the naris is formed by the septomaxilla, which is trapezoid in shape and considerably larger than in larval *E. lativittatus*. The dorsal aspect of the skull is also dominated by the nasal, frontal and parietal bones. Nasal and frontal are similar in general shape to that of larval *E. lativittatus*, apart from a less distinct anterolateral process of the nasal. The parietal, however, lacks the posterolateral process and has a gently rounded posterior edge instead of the almost straight posterior edge seen in larval *E. lativittatus*. There is no continuous bony bridge between the otic capsules,

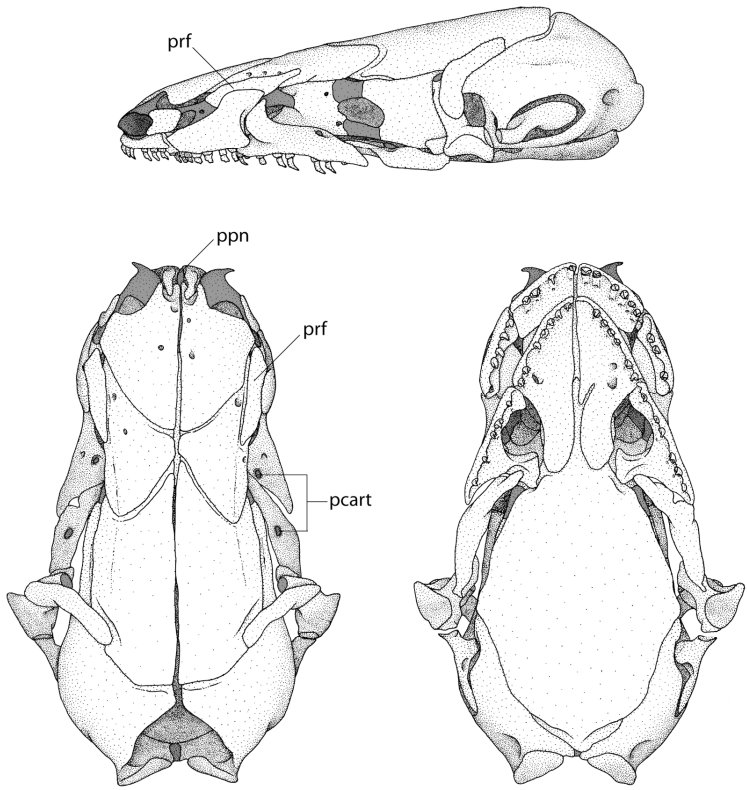

Fig. 4. Larval skull of *Ichthyophis bannanicus* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bone is stippled and cartilage stippled with with grey shading. pcart, palatal cartilages; ppn, prenasal process; prf, prefrontal. Scale bar equals 1 mm.

posterior of the parietals and dorsal of the foramen magnum. Instead, bony shelves of the dorsomedial margins of the otic capsules converge towards the dorsal midline but leave a suture between them, which forms the posterior extension of the mid-dorsal suture.

Premaxilla and maxilla are similar in general shape to that of larval *E. lativittatus*. The orbital process of the maxilla, however, does not extend as far dorsal as in *E. lativittatus* and does not overlap the frontal. Instead, it overlaps part of the dorsal side of the prefrontal, which is absent in rhinatrematids. The prefrontal forms an elongated bone on the anterolateral side of the skull, ventral

to the anterior half of the frontal, which it overlaps dorsally. The dorsolateral process of the palatine is broader than in larval *E. lativittatus* and extends further dorsal, almost reaching the same level as the dorsal tip of the orbital process of the maxilla. Further posterior, on the dorsal side of the palatine is a small depression, which accommodates a small palatine cartilage. A second, slightly larger palatine cartilage is further posterior, about half way between the first palatine cartilage and the quadrate. The second palatine cartilage lies in a shallow depression on the dorsal side of the pterygoid. Ventrally, vomer and palatine have a similar shape as in larval *E. lativittatus*. The posteromedial process of the vomer extends further posterior and the border of the choana is almost completely formed by vomer and palatine. There is, however, some variation in the examined material that indicates both intra- and interspecific variation in regard to this character. The pterygoid is smaller than in larval *E. lativittatus* and lacks the dorsal process that invests the medial side of the quadrate. The dorsal overlap of the pterygoid with the palatine is also less pronounced, although the area of contact between the palatine and pterygoid seems to have shifted somewhat towards the medial process of the palatine, which has a shallow fossa that accommodates a process of the pterygoid ventrally. The lateral side of the pterygoid forms a relatively inconspicuous pterygoid flange.

The quadrate has a slightly more slender and more obliquely oriented main body and a well developed, large pterygoid process that overlaps the pterygoid dorsally. A depression for the articulation with the anterolateral process of the stapes is present at its posterior side and is similar to that of *E. lativittatus*. The articulation for the lower jaw is similar too, although the articular facet is not as deeply notched. The dorsal-most tip of the quadrate remains cartilaginous. The squamosal is similar in shape to that of larval *E. lativittatus* and covers the lateral side of the dorsal half of the quadrate. The dorsal process of the squamosal fits a shallow fossa on the posterolateral side of the parietal, to which it is bound by connective tissue. The stapes is also similar in general shape to that of larval *E. lativittatus*, but has a more slender footplate that is separated by a relatively wide gap from the margin of the oval fenestra. The parasphenoid is separate from the

posterior endocranium and forms a large, oval plate of bone that has a narrow rostral process that extends dorsally of the vomer to about the level of the central nerve foramen of the vomer. The lateral wall of the posterior neurocranium, medial to the quadrate and immediately anterior of the otic capsule, is unossified and forms a large antotic foramen. Otherwise, the general morphology of the endocranial skeleton is similar to larval *E. lativittatus*.

The retroarticular process of the lower jaw is almost level with the rest of the lower jaw and has a length of about one third of the total length of the lower jaw. A coronoid tooth row is present and about half as long as the dentary tooth row.

Adult *Ichthyophis* also have a more robust and broader skull than adult *E. lativittatus*. The anterior copula forms a simple cap-like structure as in adult *E. lativittatus*. Nasal, premaxilla and septomaxilla are tightly sutured with each other and border the anterior copula. The septomaxilla is sutured with the prefrontal posteriorly, which prevents a contact between the maxillopalatine and the nasal. Maxilla and palatine are fused into the maxillopalatine and especially the maxillary part has expanded posteriorly, although not as extensive as in adult *E. lativittatus*. The orbital shelf of the maxillary part of the maxillopalatine has an extensive suture with the prefrontal dorsally and forms the anterior and ventral border of the orbital region. At its posterior end, the orbital shelf borders the squamosal. The actual orbit is formed by the circumorbital bone, which sits between the maxillopalatine and the sqamosal and almost completely surrounds the eye. Anteroventrally of the orbit is the tentacular groove, in which the tentacle lies. The tentacular groove is less extensively roofed as in adult specimens in recently metamorphosed and juvenile specimens. The ventral view of vomer and palatine part of the maxillopalatine is in general similar to that of larval *Ichthyophis*. The pterygoid is shorter and broader than in larvae and its lateral edge is developed into a slightly more pronounced pterygoid flange than in larvae. Anteriorly, the pterygoid is sutured to the posteromedial edge of the maxillopalatine.

The quadrate is somewhat broader and has a slightly more oblique orientation than in larvae. A relatively short quadratojugal process is present. The squamosal covers the entire dorsal part of the lateral side of the quadrate and is greatly expanded anteriorly, reaching the prefrontal. The squamosal forms the posterior and dorsal border of the orbital region and its dorsal edge is in close proximity to the frontal and parietal dorsally. A small gap between the squamosal and the parietal is usually present in juveniles but becomes progressively narrower during further ontogeny and is fully closed in adult specimens. The dorsal process of the squamosal is completely absent. The parasphenoid is fused with the posterior endocranium into the os basale.

The retroarticular process of the lower jaw is proportionately shorter than in larvae. It is further gently curved dorsally. The length of the coronoid tooth has slightly expanded to about two thirds of the length of the dentary tooth row.

Uraeotyphlus* cf. *narayani* and *U. oxyurus

The investigated larvae are very similar in their skull morphology and the following description applies to both species. The larval skull (Fig. 5) is relatively slender and elongated and the palatine-pterygoid arcade is far less prominent in dorsal view than in the larvae of *Epicrionops* or *Ichthyophis*. The anterior copula has the characteristic anterolateral wings seen in larvae of *Epicrionops* and *Ichthyophis*. The septomaxilla, however, is excluded from the border of the external naris, which is formed exclusively by the anterior copula. Medial between the anterior copulae is a long and slender, laterally compressed cartilaginous prenasal process that extends anteriorly beyond the premaxilla. The anteromedial process of the nasal extends to the anterior tip of the snout. The anterolateral process of the nasal is not very pronounced. The frontal is similar in shape to that of larval *Ichthyophis* but has a very sharply pointed posterolateral tip. The parietal is similar in shape to that of larval *Ichthyophis*. The bony shelves forming the dorsal margin of the foramen magnum are relatively narrow and have no overlap with the parietals.

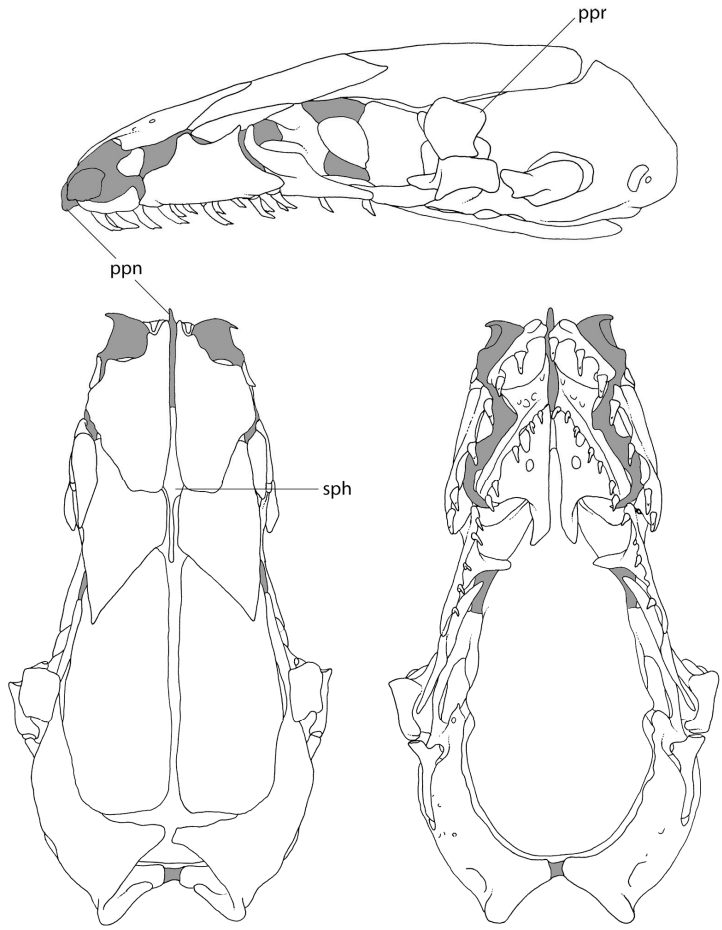

Fig. 5. Larval skull of *Uraeotyphlus* cf. *narayani* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bone is stippled and cartilage stippled with grey shading. ppn, prenasal process; ppr, posterior process of squamosal; sph, sphenethmoid. Scale bar equals 1 mm.

The premaxilla is similar to that of larval *Ichthyophis*, although the alary process does not extend as far dorsal as in larval *Epicrionops* or *Ichthyophis* and is only just about visible in dorsal view. The maxilla however, has a much broader, bicornute orbital process compared to larval *Epicrionops* or larval *Ichthyophis* and also extends further posteriorly. The overlap of the maxilla with the prefrontal is less extensive than in larval *Ichthyophis* and only the posterior tip of the orbital process overlaps the prefrontal slightly. The dorsolateral process of the palatine is also comparatively larger than in larval *Ichthyophis* and reaches the

same level as the orbital process of the maxilla dorsally, slightly overlapping the prefrontal. Premaxilla and maxilla have few, widely spaced but comparatively large, bicusped teeth. The vomer has a large, plate-like process anterior of the vomerine tooth row that partly overlaps the palatal shelf of the premaxilla dorsally. The posteromedial process is relatively small and slender compared to larval *Epicrionops* and especially larval *Ichthyophis*. The medial process of the palatine is relatively simple and slender and narrowly overlaps the parasphenoid ventrally. The pterygoid is also smaller and more slender than in larval *Ichthyophis* but otherwise of similar general shape. It has some loose contact with the pterygoid, which it narrowly overlaps dorsally. Palatal cartilages are absent.

The quadrate is very similar to that of larval *Ichthyophis* but is slightly more oblique in its orientation. A well developed pterygoid process is present and overlaps the pterygoid dorsally. The dorsal-most tip of the quadrate is cartilaginous. The squamosal is a trapezoid shaped bone that covers the lateral aspect of the dorsal half of the quadrate but does not extend much onto the dorsal side of the skull. A dorsal process is absent but a broad, relatively short posteriorly directed process is present. The squamosal is bound to the taenia marginalis part of the neurocranium dorsally but has no contact with the parietal. The stapes is similar in general shape and its articulations to that of larval *Ichthyophis*, but lacks the foramen for the stapedial artery. The parasphenoid is separate and similar in shape to that of larval *Ichthyophis*, although somewhat more slender throughout. It has a fairly inconspicuous lateral notch medial to the quadrate, which accommodates the carotid artery. A large antotic foramen is present in the lateral wall of the posterior neurocranium. Otherwise, the general morphology of the endocranial skeleton is similar to larval *Ichthyophis*.

The retroarticular process of the lower jaw is slightly bent dorsally level with the rest of the lower jaw and has a length of about one quarter of the total length of the lower jaw. A coronoid tooth row is present and about half as long as the dentary tooth row.

The adult skull (Fig. 6) is similar in overall shape to that of adult *Ichthyophis*, although the sutures between most bones are not as rigid as in

Ichthyophis. The anterior copula is still visible to a large extent, especially dorsally. The naris is relatively small and dorsolaterally directed. The cartilaginous prenasal process is still very prominent. The nasal has only a relatively narrow suture with the alary process of the premaxilla and is only loosely sutured with the septomaxilla. The sutures between the orbital shelf of the maxillopalatine and the nasal and especially the prefrontal and the nasal are also relatively wide. Nasal, frontal and parietal are relatively loosely sutured with their antimeres, leaving a narrow gap medially between the elements. Maxilla and palatine are fused and the orbital shelf of the maxillary part of the maxillopalatine has a similar extent as in adult *Ichthyophis*. The contact with the circumorbital and the squamosal, however, is less rigid. An anteroventrally oriented foramen for the tentacle is present at the anterior end of the maxillopalatine. The vomer is similar in general shape to that of the larva, except for the more expanded posteromedial process. The medial process of the palatine part of the maxillopalatine has an anteromedial extension, which, together with the vomer, forms the posteromedial border of the choana.

The quadrate has a posterior directed process that overlaps the anterolateral process of the stapes dorsally. The articulation of the lower jaw seems slightly dorsally displaced, rather than ventrally oriented as in the larvae. As in adult *Ichthyophis*, the squamosal has expanded anteriorly and has some loose contact with the maxillopalatine and the circumorbital. A small, medially directed ridge articulates medially with the posterior end of the maxillopalatine. The ventral edge of the squamosal is slightly concave. The posterior directed process of the squamosal is still present but somewhat smaller than in the larva. A relatively deep dorsomedial notch is present on the squamosal (best visible in dorsal view, see Fig. 6), which is partly responsible for the large temporal gap between the squamosal laterally and frontal and parietal medially. The parasphenoid is fused into the os basale.

The retroarticular process of the lower jaw is relatively short, only about a quarter of the total length of the lower jaw, but strongly curved dorsally. The dorsally curved part of the lower jaw does also include the articular facet. A

coronoid tooth row is present and about one quarter as long as the dentary tooth row.

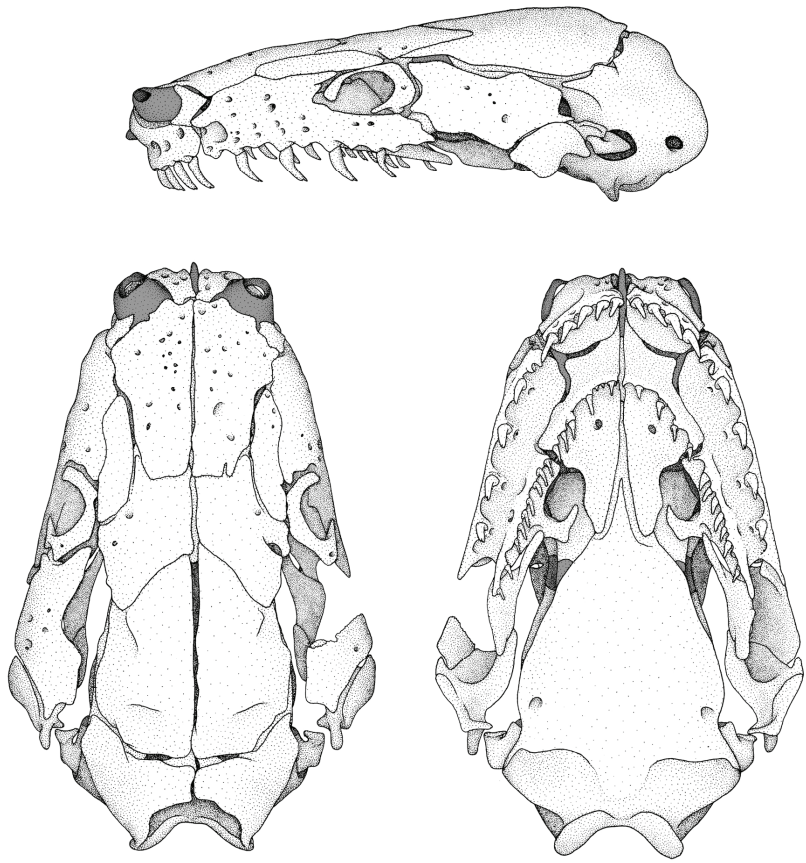

Fig. 6. Adult skull of *Uraeotyphlus* cf. *narayani* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bone is stippled and cartilage stippled with grey shading. ppn, prenasal process; ppr, posterior process of squamosal; sph, sphenethmoid. Scale bar equals 1 mm.

Praslinia cooperi

The overall shape of the larval skull (Fig. 7) is superficially similar to that of larval *Ichthyophis*. The anterior copula is covered by the nasal to a larger extent than in larvae of aforementioned taxa. The typical anterolateral wings of the anterior copula seen in other larvae are also present. A small, rounded

cartilaginous prenasal process projects slightly beyond the premaxilla. The anteromedial process of the nasal is relatively broad and short and a distinct anterolateral process is absent. The frontal is similar in general shape to that of larval *Ichthyophis*. The parietal has a sharp posterolateral corner rather than the more rounded posterior margin seen in larval *Ichthyophis* and *Uraeotyphlus*, a conspicuous process as seen in larval *Epicrionops*, however, is absent. Nasal and frontal have only a small overlap medially but are otherwise separated by a broad gap that exposes part of the cartilaginous nasal capsule. Frontal and parietal have a broad overlap. A medial gap is present between the frontals, which exposes part of the sphenethmoid.

The premaxilla is much larger compared to larval *Epicrionops*, *Ichthyophis* or *Uraeotyphlus* and extends further posteriorly. The alary process of the premaxilla is small and does not extend onto the dorsal side of the skull. The septomaxilla is very long and slender and extends parallel along the ventral edge of the nasal. Maxilla and palatine are fused into the maxillopalatine, although the general morphology of the constituent parts is more larval than adult. The maxillary part has an anterior directed process that broadly overlaps with the premaxilla laterally. A small, dorsally directed process of the orbital shelf is sutured with the frontal, and a larger, more posterodorsally directed process overlaps the frontal slightly more posteriorly. At the base of the posterodorsal process is a groove that articulates with a slender, anteroventrally directed cartilaginous process of the orbitonasal lamina. The maxillopalatine is very similar to the separate maxilla and palatine of larval *Ichthyophis*, when seen from ventral. The medial process of the palatine part of the maxillopalatine is relatively large and anteromedially extended. The choana is compeletely bordered by the palatine part of the maxillopalatine and the vomer, with the maxillopalatine forming the lateral, posterior and posteromedial border, and its anteromedial border formed by the vomer. The vomer is stouter compared to that of larval *Epicrionops*, *Ichthyophis* or *Uraeotyphlus*, and especially its posteromedial process is very broad.

The quadrate is large and has a broad corpus, compared to that of larval *Epicrionops*, *Ichthyophis* or *Uraeotyphlus*. A long pterygoid process is present and overlaps the maxillopalatine dorsally. A conspicuous anterolateral process is present on the pterygoid process and seems to articulate with the posterior-most tip of the maxillopalatine.

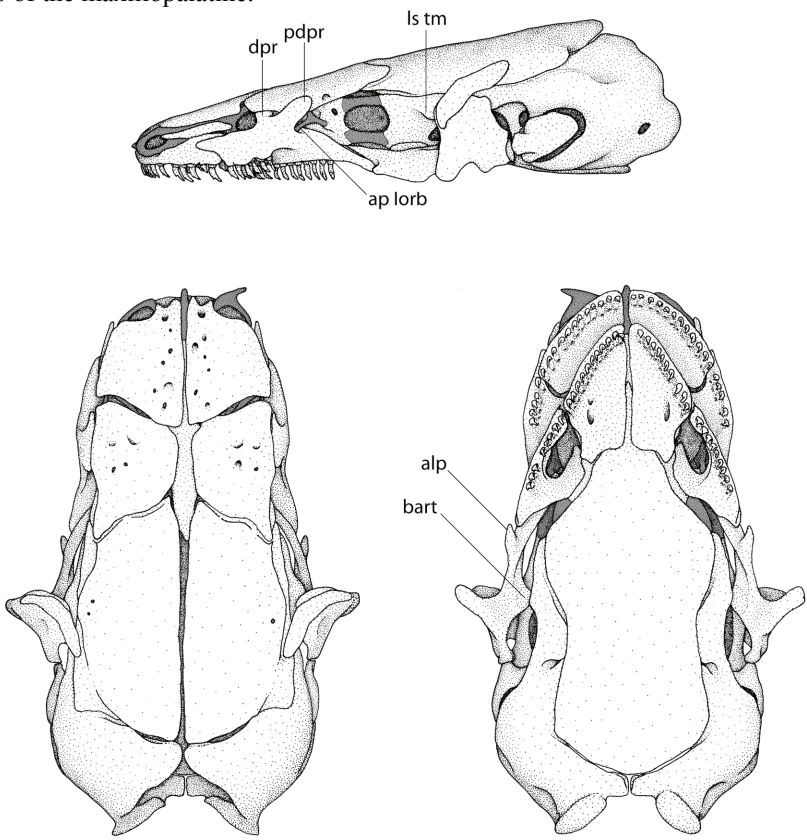

Fig. 7. Larval skull of *Praslinia cooperi* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bone is stippled and cartilage stippled with grey shading. alp, anterolateral process of pterygoid process of the quadrate; ap lorb, anteroventral process of orbitonasal lamina; bart, basal articulation; ls tm, lateral shelf of taenia marginales part of os basale; pdpr, posterodorsal process of maxillopalatine; dpr, dorsal process of maxillopalatine. Scale bar equals 1 mm.

The ventrolateral edge of the pterygoid process is slightly expanded and serves as the site of origin for the *m. pterygoideus*. The medial edge of the pterygoid process has a slight medial process that bears a facet or the articulation with the

os basale. The squamosal is comparable in shape to that of larval *Epicrionops* and *Ichthyophis*, but smaller and less strongly curved. It is lateral to the anterodorsal corner of the quadrate and extends posterodorsally onto the parietal, to which it is bound by connective tissue. General shape and articulations of the stapes are similar as in the other larvae, the footplate however, is slightly broader and shorter and also the anterolateral process is shorter than in larval *Epicrionops*, *Ichthyophis* or *Uraeotyphlus*. The parasphenoid is separate and is broadest just posterior of the maxillopalatine. Posterior to this position, it is constricted laterally and again slightly broadened just underneath the otic capsules. The rostral process of the parasphenoid extends dorsal to the vomer beyond the level of the central nerve foramen of the vomer. A large antotic foramen is present medial to the quadrate. The taenia marginalis part of the endocranium has further a well developed lateral shelf that overhangs the antotic foramen.

The retroarticular process of the lower jaw is relatively slender and gently curved dorsally. Its length is about two fifths of the total length of the lower jaw. A coronoid tooth row of about half the length of the dentary tooth row is present.

The adult skull (Fig. 8) is very broad and comparatively flat, and is superficially very similar to that of adult *Ichthyophis*. The anterior copula is simple and has no conspicuous anterolateral wing. Nasal, premaxilla and presumably also the septomaxilla are fused into the nasopremaxilla, which completely surrounds the anterior copula. Dorsally, nasopremaxilla and frontal seem proportionately larger than in the larva and are similar in size to the parietal. The frontal and especially the parietal have a laterally directed shelf that overhangs the lateral wall of the neurocranium. A small part of the sphenethmoid is still exposed between the frontals and parietals. The maxillopalatine is similar in general shape to that of adult *Ichthyophis* and *Uraeotyphlus* and is sutured with the nasopremaxilla anteriorly and anterodorsally, the frontal dorsally and the squamosal posteriorly. A shallow fossa at the anteroventral margin of the orbit accommodates the tentacle. Ventrally, the vomer is comparatively broader and more rounded anteriorly than in the larva. The medial process of the palatine part

of the maxillopalatine is raised and somewhat crest-like. The area just anterior of the choana is also slightly raised. Nasopremaxilla, maxillopalatine and vomer bear a large number of small, densely placed bicusped teeth. A pterygoid-like element of triangular shape is present posterior to the maxillopalatine, lateral of the pterygoid process of the quadrate. The pterygoid-like element articulates with the maxillopalatine, lateral to the palatine tooth row and has also some loose contact with the medial process of the squamosal.

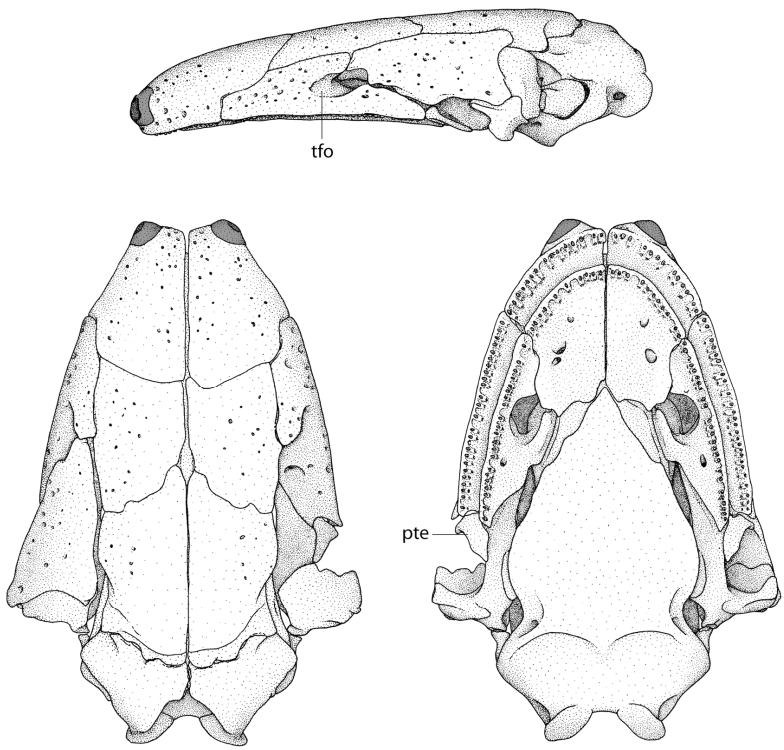

Fig. 8. Adult skull of *Praslinia cooperi* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bone is stippled and cartilage stippled with grey shading. pte, pterygoid-like element; tfo, tentacular fossa. Scale bar equals 1 mm.

The quadrate is similar in general shape to that of the larva. The anterolateral process of the pterygoid process, just posterior of the maxillopalatine, is absent. The medial articulation with the os basal is also more developed than in the larva. The squamosal has greatly expanded anteriorly and

forms the posterior border of the orbit. It is in close proximity to the frontal and parietal dorsally and leaves only a very narrow temporal gap. A small medially directed process on the anteroventral end of the squamosal articulates with the maxillopalatine. The ventral edge of the squamosal is also slightly concave as in adult *Ichthyophis* and *Uraeotyphlus*. The stapes is similar in general shape to that of the larva. The parasphenoid is fused to the os basale. The rostral process is comparatively shorter than in the larva and does not extend beyond the anterior level of the choana and has further no overlap with the vomer. Ventrally, the os basale has a pronounced lateral constriction at the level of the jaw articulation.

The retroarticular process of the lower jaw is comparatively massive and is about one third of the total length of the lower jaw. The retroarticular process is relatively straight just posterior of the articular facet, the posterior half of the process however, is approximately 25 curved dorsally. A row of small coronoid teeth is present and of about half the length of the dentary tooth row.

Sylvacaecilia grandisonae

The preservation of the cranial cartilages was poor in the examined specimens, which prevents a detailed description of the morphology of the nasal capsule and other cartilages. In the larva (Fig. 9), a very small, anteromedial process of the nasal is present and extends ventrally, medial of the alary process of the premaxillary. Nasal and frontal are of similar size, and the parietal about twice as large. Frontal and parietal are simple elements and appear almost rectangular in dorsal view. The nasals are in contact medially and frontals and parietals are only separated by a very narrow suture that partially exposes the sphenethmoid. A somewhat broader suture separates the membrane bone of the otic capsule.

The premaxilla is similar in size to that of larval *Praslinia*. The alary process of the premaxilla is small and does not extend onto the dorsal side of the skull. The septomaxilla is more elongated than in larval *Epicrionops*, *Ichthyophis* and *Uraeotyphlus*, but not as long and slender as in larval *Praslinia*. Maxilla and palatine are separate. The maxilla is similar in general shape to the maxillary part of the maxillopalatine in larval *Praslinia*, apart from a differently shaped dorsal

process, which is characterized by anterior and posterior indentations that give it a distinctly anvil-like shape. The dorsal process has only a very slight overlap with the frontal. A relatively inconspicuous dorsolateral process of the palatine abuts the posterior edge of the maxilla. The dorsolateral process does not extend dorsally as in larval *Ichthyophis* and *Uraeotyphlus*. The medial process of the palatine is relatively large and anteromedially extended, like in larval *Praslinia*. The borders of the choana are further very similar to that of larval *Praslinia*. The vomer is very similar to that of larval *Epicrionops* and *Ichthyophis*.

The corpus of the quadrate is relatively slender and has a similar orientation like in larval *Ichthyophis*. The pterygoid process is extremely long but otherwise very similar to larval *Praslinia*. It overlaps the maxillopalatine dorsally and has also a conspicuous anterolateral process that abuts the posterior-most tip of the maxillopalatine on one side. On the other side, however, is a separate element similar in size and orientation to the anterolateral process of the pterygoid process on the opposing side. This element is reminiscent of the pterygoid-like element in adult *Praslinia*. The ventrolateral edge of the pterygoid process is also slightly expanded and the medial articulation with the os basale is prominently developed. The squamosal is small and only covers the dorsal third of the quadrate laterally. The squamosal is roughly pear-shaped, with the slender end pointing downwards. It has no direct contact with either the parietal or dorsal endocranium but seems to be connected by a short tendon to the taenia marginalis part of the endocranium. The general shape and articulations of the stapes are very similar to that of the *Praslinia* larva. The parasphenoid is separate and also very similar in shape to that of the *Praslinia* larva. The rostral process of the parasphenoid overlaps part of the vomer dorsally. A large antotic foramen is present medial to the quadrate. The posterior endocranium has furthermore well-developed lateral flanges for the articulation with the pterygoid process of the quadrate.

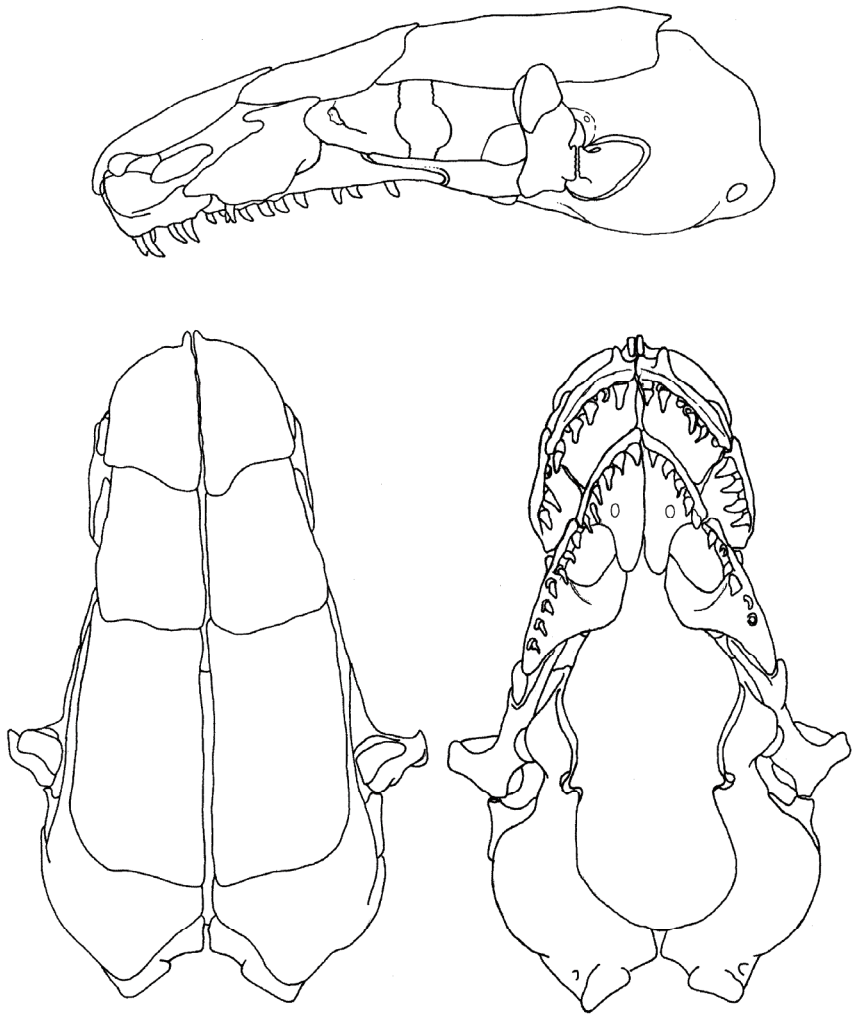

Fig. 9. Larval skull of *Sylvacaecilia grandisonae* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bony elements are stippled. Scale bar equals 1 mm.

The retroarticular process of the lower jaw is relatively massive and straight. Its length is about two fifths of the total length of the lower jaw. A short coronoid tooth row of about one sixth the length of the dentary tooth row is present.

The available adult-like skull is of a juvenile specimen (Fig. 10). Nasal, premaxilla and presumably also the septomaxilla are fused into the nasopremaxilla. The size of the nasal part of the nasopremaxilla, frontal and parietal are similar to the larva and the elements are in close contact along the dorsal midline. The frontal and parietal have a relatively narrow, laterally directed shelf that overhangs the lateral wall of the neurocranium. Maxilla and palatine are fused. The maxillopalatine is similar in general shape to that of adult *Ichthyophis* and *Uraeotyphlus* and is sutured with the nasopremaxilla anteriorly and anterodorsally, the frontal dorsally and the squamosal posteriorly. A tentacular groove is present. Ventrally, the vomer is very similar to that of the larva. The medial edges of the vomers, however, diverge posteriorly. The posterolateral edge of the vomer, which borders the choana, is slightly raised, as in *Praslinia*. A small, pterygoid-like element is present on both sides, posterior to the maxillopalatine and lateral of the pterygoid process of the quadrate, in a similar position as the anterolateral process of the pterygoid process. As in *Praslinia*, the pterygoid-like element articulates with the maxillopalatine, lateral to the palatine tooth row.

The corpus of the quadrate is broader compared to that of the larva and a short quadratojugal process is present. The medial articulation with the os basal is similarly well developed as in the larva. The squamosal has greatly expanded anteriorly and forms slender processes dorsal and ventral of the eye, thus bordering the orbit dorsally, posteriorly and ventrally. The squamosal is in close proximity to the frontal and parietal dorsally and leaves only a very narrow temporal gap. A small medially directed process is present on the anteroventral end of the squamosal and articulates with the maxillopalatine. The stapes is similar in general shape to that of the larva. The parasphenoid is fused to the os basale. The os basale has well developed lateral processes that articulate with the retroarticular process of the quadrate and further has a pronounced lateral constriction at the level of the quadrate, as seen in *Praslinia*.

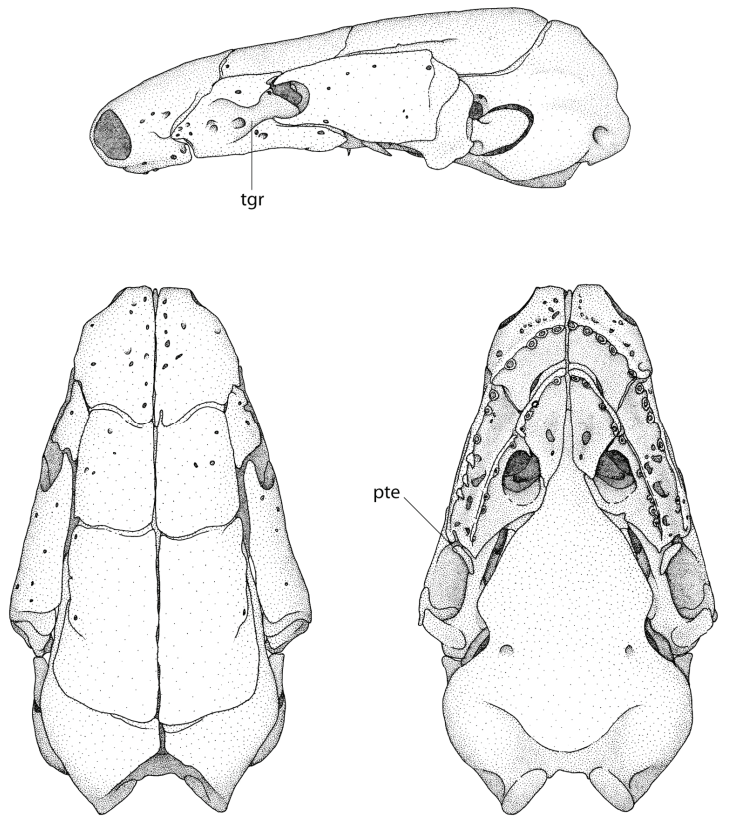

Fig. 10. Adult skull of *Sylvacaecilia grandisonae* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bony elements are stippled. pte, pterygoid-like element; tgr, tentacular groove. Scale bar equals 1 mm.

The retroarticular process of the lower jaw is comparatively massive and relatively straight. A coronoid tooth row is present and has about one quarter of the length of the dentary tooth row.

Grandisonia* cf. *larvata* and *G. sechellensis

The following description applies to both species, which have a very similar general skull morphology except where noted. The larval skull is very slender and elongated (Fig. 11). The anterior copula has the characteristic anterolateral wings seen in other larval caecilians. The external naris is bordered exclusively by the

anterior copula. Medial between the anterior copulae is a long and thin cartilaginous prenasal process that is about level with the premaxilla anteriorly. The anteromedial process of the nasal is relatively short and slender and extends to the anterior tip of the snout. The anterolateral process of the nasal is not very pronounced. Nasal, frontal and parietal are similar in shape to that of larval *Sylvacaecilia*, except for a more pronounced medioposterior process of the nasal and a small posterolateral process of the parietal. Nasal, and especially the frontal and parietal are relatively widely separated from their antimeres and the sphenethmoid is exposed between them. The bony shelves forming the dorsal margin of the foramen magnum are widely separated and have also no overlap with the parietals.

The premaxilla is similar in size and extent to that of larval *Praslinia*. The alary process of the premaxilla is relatively small and does not extend far dorsally. The septomaxilla is very long and extremely slender and extends parallel along the ventral edge of the nasal. Maxilla and palatine are fused into the maxillopalatine, although the general morphology of the constituent parts, as in larval *Praslinia*, is more larval than adult. The maxillary part has an anteriorly directed process that broadly overlaps with the premaxilla laterally. A broad orbital process overlaps the frontal. At the base of the posterodorsal process is a groove that articulates with a slender, anteroventrally directed cartilaginous process of the orbitonasal lamina, similar to larval *Praslinia*. The maxillopalatine is very similar to the separate maxilla and palatine of larval *Ichthyophis* and *Praslinia*, when seen from ventral. The medial process of the palatine part of the maxillopalatine is relatively long and slender and anteromedially extended. The choana is bordered by the vomer anteromedially and the palatine part of the maxillopalatine laterally, posteriorly and posteromedially. The vomer is relatively similar to that of larval *Sylvacaecilia*, although its posteromedial process is slightly broader and shorter.

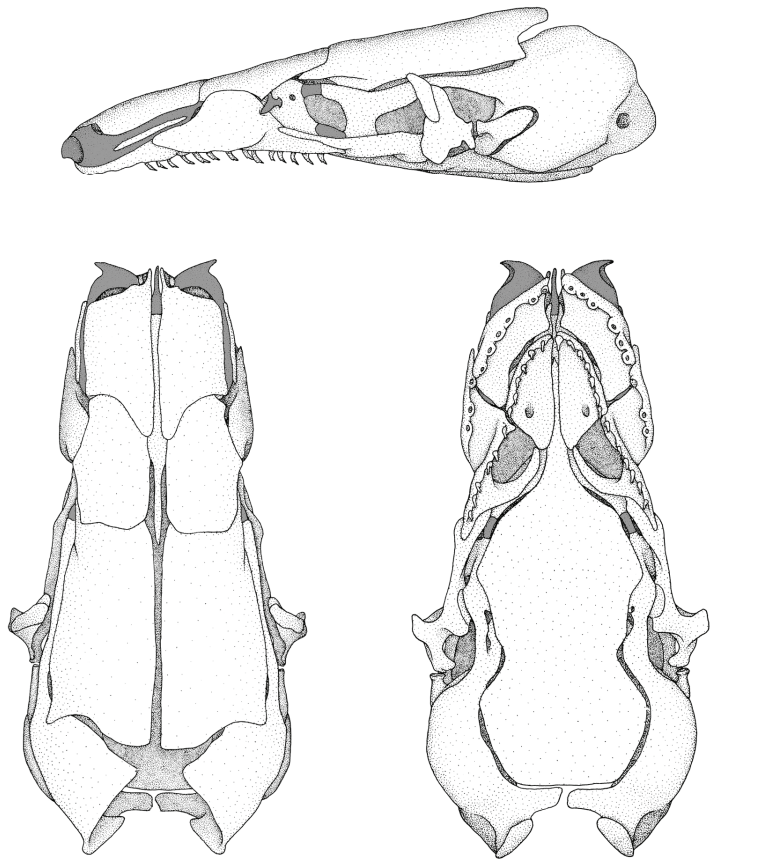

Fig. 11. Larval skull of *Grandisonia sechellensis* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bone is stippled and cartilage stippled with grey shading. Scale bar equals 1 mm.

The quadrate is very similar to that of larval *Sylvacaecilia*, including the extremely elongated pterygoid process, which broadly overlaps the maxillopalatine dorsally. The medial edge of the retroarticular process has a slight medial process that bears a facet for the articulation with the os basale. The squamosal is small and forms a simple elongated bony plate that is slightly obliquely oriented. It is lateral to the anterodorsal corner of the quadrate and extends dorsally, but does not articulate with the parietal or endocranium. The footplate of the stapes is narrower than in the other larval caecilians and the oval

foramen seems to be in a more anteromedial position than in the other larval forms. The parasphenoid is separate and has a pronounced lateral constriction and a very long and slender rostral process, which give the parasphenoid a violin-like shape. A very large antotic foramen is present medial to the quadrate, leaving most of the lateral wall of the braincase unossified, apart from the taenia marginalis and pila antotica.

The retroarticular process of the lower jaw is relatively slender and slightly curved dorsally. Its length is about one third of the total length of the lower jaw. A coronoid tooth row of about one third the length of the dentary tooth row is present.

The adult skull (Fig. 12) is also very elongated and pointed and resembles a slightly stretched adult *Sylvacaecilia* skull. The anterior copula is simple and has no conspicuous anterolateral wing. Nasal, premaxilla and presumably also the septomaxilla are fused into the nasopremaxilla, which completely surrounds the anterior copula. A cartilaginous prenasal process is visible between the nasopremaxillae but does not extend beyond these. Dorsally, nasopremaxilla and frontal seem to be of similar size, with the parietal being slightly larger. The frontal and parietal have a relatively narrow, laterally directed shelf that overhangs the lateral wall of the neurocranium. The sphenethmoid is still exposed between the frontals and also the nasals anteriorly. The maxillopalatine is similar in general shape to that of adult *Praslinia* and *Sylvacaecilia* and is sutured with the nasopremaxilla anteriorly and anterodorsally, the frontal dorsally and the squamosal posteriorly. Ventrally, the vomer is slightly more elongate than in the larva and the medial edges of the vomers diverge posteriorly. The palatal shelf of the palatine part of the maxillopalatine anterior to the choana is expanded and excludes most of the vomer from the anterior and anteromedial border of the choana.

The corpus of the quadrate is slightly broader compared to that of the larva and a short quadratojugal process is present. The squamosal has greatly expanded anteriorly and also ventrally and forms and borders the orbit posteriorly. The squamosal is in close proximity to the frontal and parietal

dorsally but leaves a narrow temporal gap. A small, medially directed process is present on the anteroventral end of the squamosal, which articulates with the maxillopalatine but also forms part of the border of the mouth. The footplate of the stapes is broader as compared to that of the larva. The parasphenoid is fused to the os basale and similar in shape to that of adult *Praslinia*. The rostral process of the parasphenoid part of the os basale does not extend as far anteriorly as in the larva and does further has no overlap with the vomer. The os basale has well developed lateral processes that articulate with the retroarticular process of the quadrate and further has a pronounced lateral constriction at the level of the quadrate, as seen in *Praslinia* and *Sylvacaecilia*.

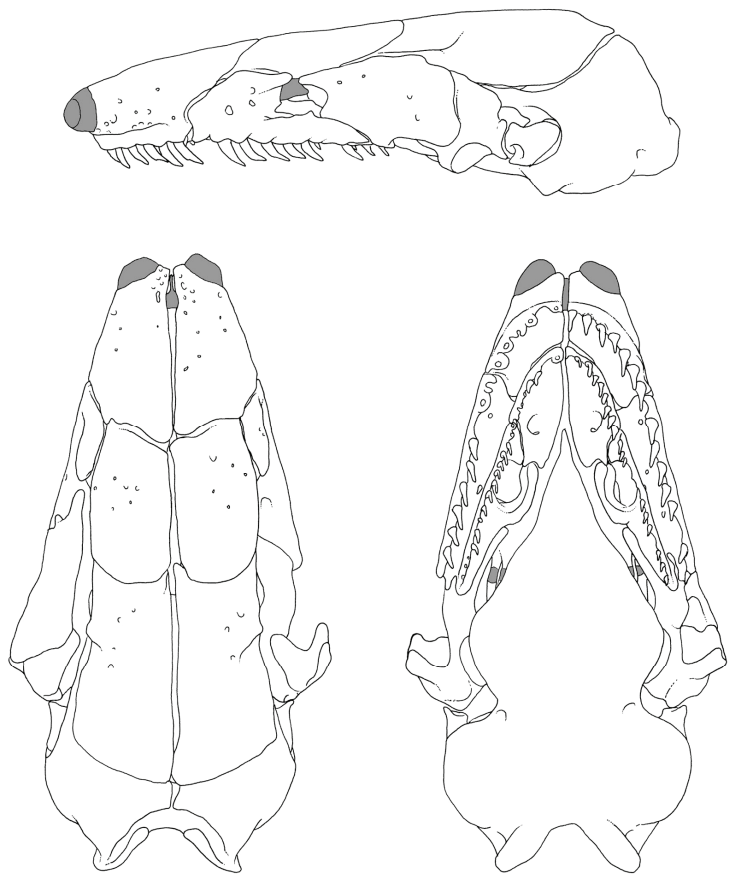

Fig. 12. Adult skull of *Grandisonia sechellensis* in lateral (top), dorsal (bottom left) and ventral (bottom right) view. Bone is stippled and cartilage stippled with grey shading. Scale bar equals 1 mm.

The retroarticular process of the lower jaw is comparatively slender but strongly dorsally oriented. A coronoid tooth row is present and has about one third of the length of the dentary tooth row.

Hyobranchial skeleton

Epicrionops lativittatus

The larval hyobranchial skeleton consist of 5 paired elements, the ceratohyals and the ceratobranchials I to IV, and unpaired medial elements, the basihyal and basibranchials I and II (Fig. 13A). The basihyal and basibranchials are cartilaginous while the ceratohyal and ceratobranchials I, II and III are largely ossified apart from their medial and distal tips, which remain cartilaginous. The distal third of ceratobranchial IV is cartilaginous while the rest of the element is ossified. The basihyal is the largest medial element, dorsoventrally compressed and pear-shaped in ventral view with the broadly rounded end anteriorly. The basihyal articulates loosely with the basibranchial I posteriorly, which forms a simple rod slightly longer than half the length of the basihyal. Basibranchial II is much smaller and shorter, about a third the size of basibranchial I. Basibranchial I and II are separated by a gap the size of basibranchial II. The longest element is the ceratohyal, which has a broad, slightly dorsoventrally compressed medial head that articulates loosely with the basihyal and basibranchial I. The ceratohyal is becoming slightly laterally compressed towards its distal end. Ceratobranchials I, II and III are of similar size each and form relatively slender bars. Ceratobranchial IV is less than half the length of ceratobranchial III, triangular in shape and distinctly dorsoventrally compressed. The medial end of ceratobranchial IV articulates with the posterior edge of ceratobranchial III in a medial position. Ceratohyal and ceratobranchial I have a relatively simple arc shape, whereas the distal ends of certobranchial II and especially certobranchial III have a more posteriorly directed curvature that is associated with the gill aperture. In a metamorphic specimen, most of the bony elements have an irregular appearance and some bony elements are fragmented into several

disconnected pieces. An apparently newly formed, thin cartilaginous rod is seen parallel to and posterior of ceratobranchial I.

In the adult specimen investigated, the hyobranchial skeleton is entirely cartilaginous and consists of ceratohyals and three ceratobranchial arches (probably representing ceratobranchials I, II and III). The ceratohyal is the largest element, dorsoventrally compressed and scythe-shaped in ventral view, while ceratobranchial I, II and II are more rod-like, with ceratobranchial I being the largest ceratobranchial and III the smallest. All arches are fused medially with their antimeres. The ceratohyal arches are also medially fused with a short and slender, unpaired medial element that is also fused with the ceratobranchial arches I. Based on the larval configuration, this element possibly represents the ceratobranchial I but might also partially incorporate the basihyal.

Ichthyophis bannanicus* and *I.* cf. *kohtaoensis

Larvae and adults of both *I. bannanicus* and *I.* cf. *kohtaoensis* have a virtually identical configuration of the hyobranchial skeleton. In larvae it is entirely cartilaginous and consists of five pairs of arches, the ceratohyals and ceratobranchials I to IV and two unpaired medial elements, the basihyal and basibranchial. The basihyal forms a dorsoventrally compressed, more or less rectangular plate that articulates with the ceratohyal posterolaterally and the basibranchial posteriorly. The basibranchial is a simple rod-shaped cartilage that slightly decreases in diameter posteriorly and articulates loosely with the ceratohyal anteriorly, the ceratobranchial I medially and ceratobranchial II posteriorly. The general configuration of the larval hyobranchial skeleton is similar to that of *Epicrionops latvittatus* in that the ceratohyal is the largest element, with the ceratobranchials I, II and II smaller, but similar in size to each other, and ceratobranchial IV triangular in shape and strongly dorsoventrally compressed. However, the ceratohyal has a different, more complicated shape compared to *E. lativittatus*. It is dorsoventrally compressed but becomes more laterally compressed distally and extends from a medioventral to a posteriolateral position for about half its length, at which point it forms a posteriorly directed

spur at its ventroposterior edge. From this position on, the ceratohyal extends posteriorly in close proximity to the retroarticular process of the lower jaw and becomes increasingly dorsoventrally compressed until it forms an almost horizontally oriented, slightly concave plate at its posterior end. The posterior end of the ceratohyal is furthermore enlarged by a relatively large flange that extends from its ventrodistal edge and roofs the distal end of ceratobranchial I, which with it articulates.

The basihyal and basibranchial are fused in a large larva of *I. bannanicus* (Fig. 13B), which shows no signs of metamorphosis otherwise. Several specimens of *I.* cf. *kohtaoensis* are at various stages of metamorphosis. In the youngest of these, basihyal and basibranchial are fused into a single element. This is followed by the shortening of the fused basihyal-basibranchial element and a remodelling of the ceratohyal, which attains a more simple rod-like shape. In juvenile specimens shortly after metamorphosis, the ceratobranchial IV is much reduced in size and forms a small medioposteriorly directed spur at the distal end of ceratobranchial III. Ceratobranchial IV is nonetheless recognizable as a separate element and does not fuse with ceratobranchial III for some time. In older juveniles, ceratobranchial IV is fused to ceratobranchial III but forms a still relatively large spur, giving the fused ceratobranchial III+IV a similar appearance as in adult *Uraeotyphlus* cf. *narayani* (see below).

The hyobranchial skeleton of adult *I. bannanicus* and *I.* cf. *kohtaoensis* is also entirely cartilaginous and consists of four arc-shaped structures. The ceratohyals are fused medially to the basihyal-basibranchial, which is also fused with the ceratobranchials I, similar to adult *E. lativittatus*. In contrast to *E. lativittatus*, all branchial arches are of similar size and all are slightly dorsoventrally compressed. The distal end of ceratobranchial III+IV is slightly expanded.

Uraeotyphlus* cf. *narayani* and *U. oxyurus

Larvae of both species of *Uraeotyphlus* investigated have a hyobranchial skeleton very similar to that of larval *Ichthyophis* examined here. The main differences are

in the proportions of some of the elements. The ceratohyal, ceratobranchials I, II and II and the basibranchial are very similar to those of larval *Ichthyophis*, but the ceratobranchial seems to be proportionately broader (Fig. 13C). The medioposterior spur and ventrodistal flange of the ceratohyal are furthermore not as pronounced as in larval *Ichthyophis*. The main difference between larval *Uraeotyphlus* and *Ichthyophis* is in the shape of the basihyal, which has a broad, relatively short process anterolaterally on each side.

The adult hyobranchial skeleton of *U.* cf. *narayani* is also similar to that of adult *Ichthyophis*, although its arches have a somewhat more slender appearance (Fig. 13D). The most pronounced difference is in the shape of the distal end of ceratobranchial III+IV, which is broader than in *Ichthyophis* and has a pronounced posteromedial directed spur that likely represents the fused ceratobranchial IV.

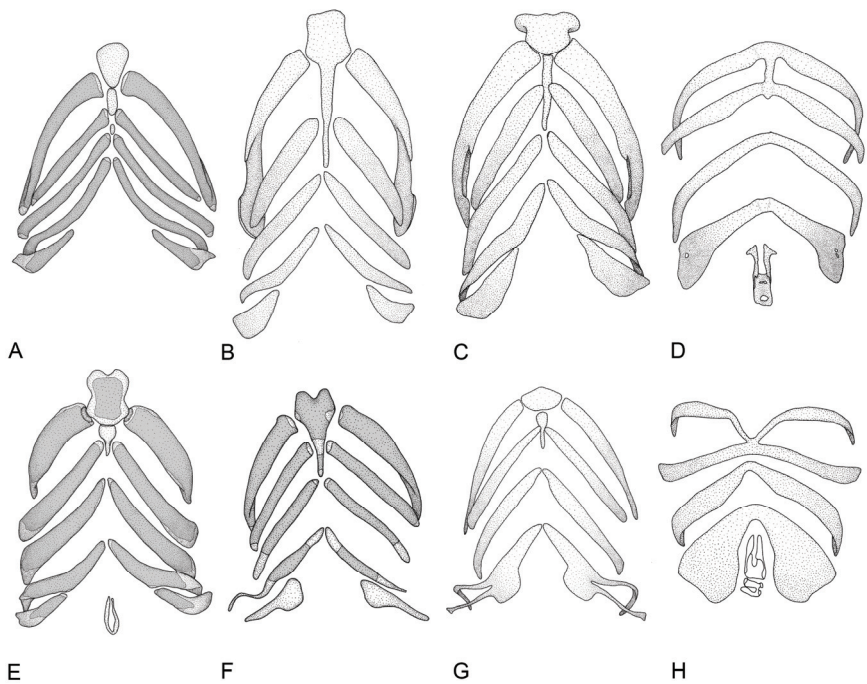

Fig.13. Hyobranchial skeleton of (**A**) *Epicrionops lativittatus* larva, (**B**) *Ichthyophis bannanicus* larva, (**C**) *Uraeotyphlus* cf. *narayani* larva, (**D**) *Uraeotyphlus* cf. *narayani* adult, (**E**) *Praslinia cooperi* larva, (**F**) *Sylvacaecilia grandisonae* larva, (**G**) *Grandisonia sechellensis* larva and (**H**) *Grandisonia sechellensis* adult. Grey areas denote bone in **A**, and mineralized cartilage in **E** and **F**. Not to scale.

Praslinia cooperi

All paired elements of the larval hyobranchial skeleton are comparatively broad (Fig. 13E). The ceratohyal is the broadest, especially medially, and ends in a rather short and blunt tip distally. Ceratobranchials I and II are more slender medially and broader distally. Ceratobranchial IV is a separate element and articulates with an inconspicuous process at the posterior edge of the medial ceratobranchial III. The basihyal is roughly rectangular, large, broad and dorsoventrally compressed with a median constriction and an anterior notch. It has short, broad posterolateral processes that articulate with a depression in the medial end of the ceratohyal. The basihyal also articulates with a small, cone-shape basibranchial posteriorly via an inconspicuous process. The basibranchial has shallow depressions medially on each side that articulate with the medial end of ceratobranchial I. All elements of the hyobranchial skeleton, except the basibranchial, show a strong retention of alizarin red. This stain is highly sensitive for bone and other calcified structures. At high magnification, the stained areas of the hyobranchial elements appear granular, with a relatively rough texture and coarse edges. This is indicative of calcified cartilage, rather than true bone, but histology is needed to establish the nature of these calcifications.

The adult hyobranchial skeleton of *Praslinia cooperi* is similar to that of adult *Ichthyophis* and *Uraeotyphlus*, except for the fused ceratobranchial III+IV, which is considerably expanded distally.

Sylvacaecilia grandisonae

The ceratohyal is the largest element of the hyobranchial skeleton in larvae (Fig. 13 F); it is broad medially and has a sharp posterior edge, similar to larval *Epicrionops lativittatus*. Ceratobranchials I and II are simple, relatively slender rods. Ceratobranchial III is characterized by a very long and thin distal end that is slightly twisted and supports the gill slit. Ceratobranchial I is triangular to club-shaped but unlike in the larval caecilians discussed above, ceratobranchial IV is broadest at its medial end and tapers towards its distal end. An unpaired, medial

element is present and articulates with the ceratohyals and ceratobranchials I and II. It is club-shaped, with a broad, anterior half that articulates with the ceratohyal and has an anteromedial notch, and a more slender, posterior portion that articulates with ceratobranchials I and II. This medial element probably represents a fused basihyal and basibranchial, but ontogenetic data are needed to verify this. Large parts of the ceratohyal, ceratobranchial I to III and the median element stain positive for alizarin red. Like in *Praslinia cooperi*, this seems to be due to extensive mineralization of the cartilage rather than true ossification. Ceratohyal and ceratobranchials I and II are completely mineralized apart from their medial- and distal-most tips. Ceratobranchial III has only its more central part mineralized, with the medial tip remaining largely cartilaginous and also the curled, distal part. The median element has an anterior mineralization that encompasses most of the broad, anterior part apart for the articulation sites with the ceratohyal, and a smaller, posterior rod-like mineralization between ceratobranchials I and II. Ceratobranchial IV is entirely cartilaginous.

The hyobranchial skeleton of the juvenile specimen is similar to that of the adult *Praslinia cooperi*. However, the fusion of ceratobranchial III and IV does not seem to be complete and ceratobranchial IV forms a broad, disc-like medial process, while the distal tip of ceratobranchial III is still distinguishable. Otherwise it has the typical shape of the adult hyobranchial skeleton seen in other caeciliid and most typhlonectid species (Nussbaum, 1977; Wilkinson and Nussbaum, 1997; Wake, 2003).

Grandisonia* cf. *larvata* and *G. sechellensis

The larval hyobranchial skeleton of *G.* cf. *larvata* and *G. sechellensis* (Fig. 13G) is superficially most similar to that of larval *Sylvacaecilia grandisonae*. A broad, dorsoventrally compressed basihyal articulates with the ceratohyal laterally and the basibranchial posteriorly. The basihyal has the shape of about one third of a circle, with the blunt tip pointing anteriorly. The basibranchial is similar in shape to that of larval *Praslinia cooperi* but somewhat more slender and elongated and articulates medially with the medial ends of ceratobranchials I. The ceratohyal is

the longest element and slightly broader medially, as in the other larvae. Ceratohyal and ceratobranchial I and II are relatively simple, slightly dorsoventrally compressed, cartilaginous rods. Ceratobranchial II and especially IV are broadened medially and have thin and long distal parts. The distal end of ceratobranchial III is strongly curved and associated with the gill aperture, around which it coils. The distal most tip of ceratobranchial IV is slightly broadened. Unlike in the other caecilian larvae investigated, ceratobranchial III and IV are fused along their medial edges.

The adult hyobranchial skeleton of *Grandisonia sechellensis* has a morphology typical of that of other adult caeciliid species (see Fig 13H).

Musculature

Epicrionops lativittatus* and *Rhinatrema bivittatum

Four major trigeminus innervated jaw adductors are present in larval *Epicrionops lativittatus*. The most lateral is the *m. adductor mandibulae externus*, a bulky and fleshy muscle that originates from the lateral edge of the quadrate, ventral to the squamosal (Fig. 14). Its fibres run in a posterodorsal to anteroventral direction and insert on the dorsolateral side of the lower jaw.

Medial to the posterior end of the *m. adductor mandibulae externus*, and separated from it by the mandibular branch of the trigeminus, is the *m. adductor mandibulae articularis*. This muscle is the smallest of the lower jaw adductors and originates from the anterior side of the quadrate. Its fibres are relatively short and insert on the dorsal and dorsolateral side of the lower jaw, between the jaw articulation and the entry of the mandibular branch of the trigeminus into the lower jaw.

Dorsal and medial to the *m. adductor mandibulae externus* is the *m. adductor mandibulae longus*, by far the largest primary adductor muscle, which dominates the head in lateral and dorsal view. It originates from the parietal, frontal, the anterior margin of the squamosal, quadrate, and the otic capsule and taenia marginalis parts of the endocranium. The *m. adductor mandibulae longus* has a complex fibre architecture and several portions can be recognized. The

largest, most lateral of these is the superficial layer, which originates from the fascia of the *m. depressor mandibulae*, the anterior margin of the squamosal and the medial part of the parietal. It has a pinnate fibre architecture and inserts on the dorsal side of the lower jaw via a strong tendon and also partly into the lip fold. The fibres of the superficial layer of the *m. adductor mandibulae longus* are not well separated from the *m. adductor mandibulae externus* near their insertion. Posteromedial to the superficial portion of the *m. adductor mandibulae longus* is a posterior portion, whose fibres originate from the lateromedial side of the parietal and the otic capsule and insert directly on the dorsal side of the lower jaw, behind the insertion of the superficial portion. The medial-most portion of the *m. adductor mandibulae longus* is the medial layer, which originates from the frontal, parietal and posterodorsal parts of the endocranium and inserts on the medial side of the lower jaw via a broad fascia. Anteriorly, just behind the eye, it reaches onto the dorsal side of the skull from where it tapers gradually towards the taenia marginalis part of the endocranium.

Medial to the *m. adductor mandibulae longus* and separated from it by the ramus maxillaris of the trigeminus is the *m. adductor mandibulae internus*. It originates from the frontal, just behind the eye, and the taenia marginalis part of the os basale and inserts on the medial side of the lower jaw via a broad fascia. It is a relatively thin sheet of muscle whose fibres are relatively long and more obliquely oriented anteriorly and increasingly shorter and vertically oriented towards its posterior end.

Posterior and slightly medial to the *m. adductor mandibulae internus* is the *m. levator quadrati*. It is a small muscle that forms a thin and relatively narrow sheet that originates from the side wall of the braincase and inserts slightly further anteriorly on the pterygoid process of the quadrate. Its fibres have an almost vertical orientation.

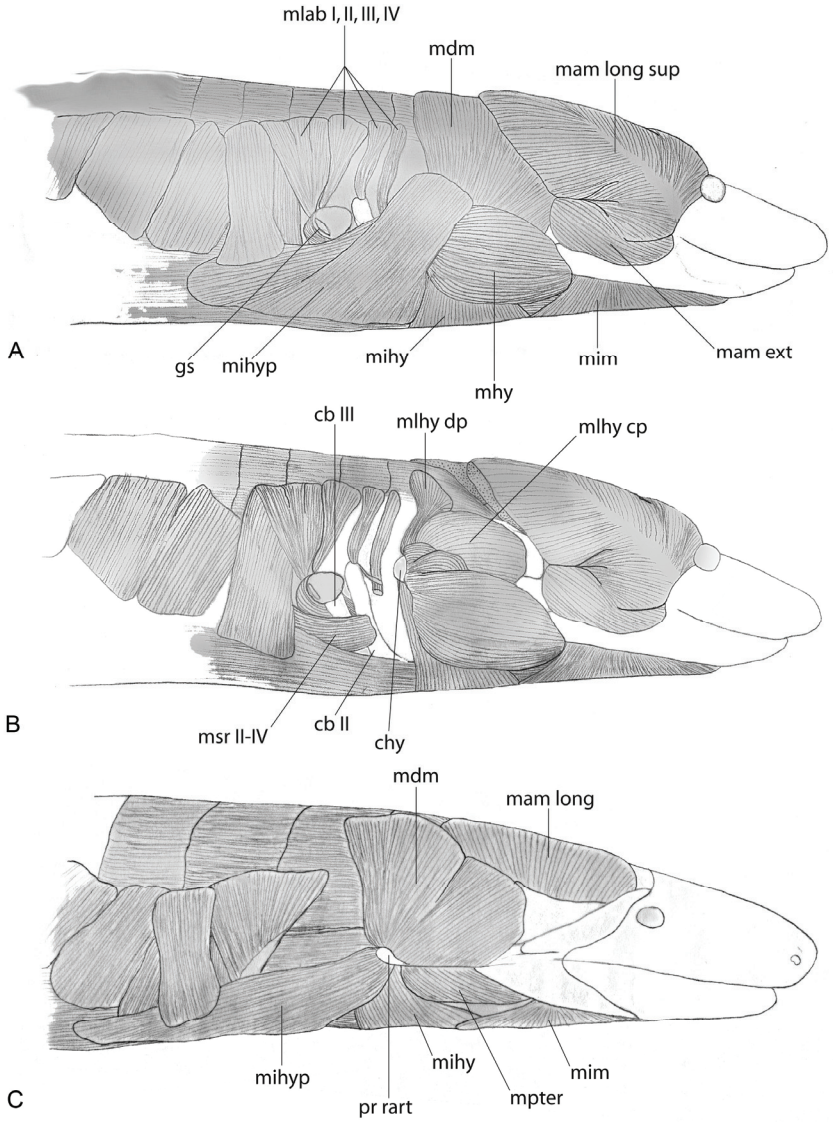

Fig. 14. Musculature of larval and adult *Epicrionops lativittatus*. **A** superficial musculature of larva. **B** Larva, with *m. depressor mandibulae* and *m. interhyoideus posterior* removed. **C** Superficial musculature of adult. cb II, ceratobranchial II; cb III, ceratobranchial III; chy, ceratohyal; gs, gill slit; mam ext, *m. adductor mandibulae externus*; mam long sup, superficial layer of *m. adductor mandibulae longus*; mam long, *m. adductor mandibulae longus*; mdm, *m. depressor mandibulae*; mihy, *m. interhyoideus*; mihyp, *m. interhyoideus posterior*; mlhy cp, cranial portion of *m. levator hyoideus*; mlhy dp, dorsal portion of *m. levator hyoideus*; mhy, *m. hyomandibularis*; mim, *m. intermandibularis*; mlab I, II, III, IV, *m. levator arcus branchialis* I, II, III, IV; mpter, *m. pterygoideus*; msr II-IV, *m. subarcualis rectus* II-IV; pr rart, retroarticular process. Not to scale.

Ventral to the *m. levator quadrati* is the origin of the *m. pterygoideus*, which is part of the trigeminus innervated muscle group. It has a broad origin from the ventrolateral edge of the pterygoid, just anterior to the jaw articulation and its fibres run in a shallow arc around the internal process of the lower jaw and insert on the medial side of the retroarticular process.

Lateral to the *m. pterygoideus* is the *m. hyomandibularis*. This is a large and fleshy muscle that originates from the ventral side of the lower jaw along its entire length from the jaw articulation to the caudal tip of the retroarticular process. Its fibres run along the ventral and lateral side of the retroarticular process and insert on the lateral side of the distal ceratohyal. Most of this muscle is externally visible, except for the posterior part that inserts on the ceratohyal, which is covered by the *m. interhyoideus posterior*.

Dorsal to the *m. hyomandibularis* and posterior to the *m. adductor mandibulae longus* is the *m. depressor mandibulae*. It is a large muscle that originates from the lateral side of the squamosal and the dorsal fascia and inserts on the dorsal side of the retroarticular process.

Medial to the *m. depressor mandibulae* is the *m. levator hyoideus*. This muscle has two distinct portions, a small, fan-shaped dorsal portion originates from the dorsal fascia and its fibres run almost vertically towards the insertion on the medial side of the distal-most tip of the ceratohyal. The second, cranial portion is much larger and is itself divided into two parts, a larger, dorsal one that has its origin from the posterior edge of the squamosal and otic capsule, and a ventral part that originates from the quadrate. The two parts are separated by a branch of the facial nerve. The ventral part has horizontally oriented fibres whereas the dorsal part has both horizontally and more obliquely oriented fibres. Both parts have their insertion on the dorsolateral, dorsal and dorsomedial side of the distal ceratohyal, with the insertion of the dorsal part being distal to that of the ventral part.

The *m. intermandibularis* is part of the trigeminus innervated musculature. It originates from the medial side of the pseudoangular, anterior of the jaw articulation and inserts in a mid-ventral fascia. The fibres of the *m.*

intermandibularis have a roughly fan-like orientation and cover almost the entire area between the mandibular rami from the symphysis to the level of the jaw articulation except for a small, triangular area just behind the symphysis. At its posterior end, its fibres diverge somewhat from their midventral insertion and overlap the m. interhyoideus anterior.

The facialis innervated *m. interhyoideus* covers the area between the ceratohyals, posterior of the *m. intermandibularis*. Its fibres originate from the ventral margin of the ceratohyal via a broad fascia and insert in a midventral fascia. Most fibres have an anteroventral orientation in contrast to the fan-shape of the *m. intermandibularis*. The posterior margin of the *m. interhyoideus* is overlapped by the *m. interhyoideus posterior*, which covers the ventrolateral side of the branchial region. Its anterior end reaches the level of the posterior end of the retroarticular process of the lower jaw. It is not attached to the retroarticular process but overlaps the posterior part of the *m. hyomandibularis* and the posteroventral part of the *m. depressor mandibulae*. Anteriorly, it forms a parallel-fibred muscle sheet that originates from a fascia overlying the *m. depressor mandibulae* and inserts in a ventral fascia. It has a posterior part with more oblique fibres, some of which originate from a short, strong tendon medial to the anterior part of the *m. interhyoideus posterior*, between the distal ends of the ceratohyal and ceratobranchial II. This tendon is also the insertion for a small branchial adductor muscle that originates from the distal tip of ceratobranchial II.

The larval *Rhinatrema bivittatum* investigated has an essentially similar topology of its superficial musculature. We did not dissect deeper layers of musculature in this specimen.

The primary jaw adductor musculature of adult *Epicrionops lativittatus* is covered by the squamosal and maxillopalatine laterally, which have expanded to partly cover the cheek region (Fig. 14C). The *m. adductor mandibulae externus* has disappeared. The *m. adductor mandibulae articularis* is in a similar position to that in the larva and maintains the same origin and insertion. The *m. adductor mandibulae longus* is still the largest primary jaw adductor but is less extensive and fleshy than in larvae. As in the larva, three layers can be distinguished

macroscopically. The largest, superficial layer extends through the gap between squamosal and maxillopalatine laterally and parietal and frontal medially onto the dorsal side of the skull, where it originates from the parietal and posteriormost part of the frontal medially and the dorsalmost part of the os basale. It inserts on the dorsal side of the skull with a strong but short tendon. Immediately caudally is a relatively small posterior layer, whose long fibres originate from the posterior part of the parietal and the anterior side of the quadrate and insert directly onto the dorsal edge of the lower jaw. The medial layer is also less extensively developed than in the larva but maintains essentially the same spatial relationships. The *m. adductor mandibulae internus* does not extend as far anterodorsally as in the larva but has otherwise a similar origin and insertion. Its anterior portion has its origin anteroventrally of the eye. As in the larvae, the *m. levator quadrati* is a small muscle with similar origin and insertion.

The *m. hyomandibularis* is no longer present, which exposes the *m. pterygoideus* in lateral view. The *m. pterygoideus* has the same origin and insertion as in larvae but is larger and appears fleshier, with a larger diameter in cross section.

The posterior part of the *m. depressor mandibulae* does not meet its counterpart in the dorsal midline and has shifted its origin to a more dorsolateral position. It also extends further anteriorly than in larvae as a result of the anterior extension of the squamosal. Its origin from the dorsal fascia and squamosal and insertion on the dorsal side of the retroarticular process, however, are the same as in larvae. Both portions of the *m. levator hyoideus* have disappeared.

The topology of the *m. intermandibularis* is similar to the larval condition. The *m. interhyoideus* is also similar in extent to that in larvae but originates solely from the retroarticular process. The *m. interhyoideus posterior* has shifted its sole origin to the distal tip of the retroarticular process, where it attaches directly to the bone. It appears narrower in lateral view and its fibres are relatively long and form a moderately fan-like muscle.

Ichthyophis bannanicus* and *I.* cf. *kohtaoensis

Unless mentioned otherwise, the following description applies to both species, which have a very similar arrangement of the cranial muscles. The arrangement of the trigeminus innervated musculature in the larvae is similar to that seen in larval *Epicrionops lativittatus*, except for obvious differences in size and proportions. The *m. adductor mandibulae externus* has the same spatial relationships as in *E. lativittatus*, but is much smaller and its fibres are more horizontally oriented. Due to its smaller size, a part of the *m. adductor mandibulae articularis* is externally visible. The *m. adductor mandibulae articularis* is a small muscle with almost vertically oriented fibres that originate from the anterior side of the quadrate and insert on the lower jaw, just in front of the jaw articulation.

The *m. adductor mandibulae longus* is the largest primary jaw adductor, as in *E. lativittatus*, but does not cover the dorsal side of the head completely (Fig. 15A). Three main, incompletely separated fibre layers can be recognized. The largest of these is the superficial layer, which partly overlaps the *m. depressor mandibulae*, and originates from the fascia of the depressor mandibuale and the mediolateral side of the parietal and frontal. It inserts on the dorsal side of the lower jaw via a broad fascia, which also attaches to the skin of the lip fold in the mouth angle. The fascia extends dorsoposteriorly within the superficial layer but is barely visible externally and far less developed compared to *E. lativittatus*. The posterior layer originates from the anterior side of the quadrate and, to a lesser extent, from the posterior taenia marginalis part of the endocranuim and inserts directly onto the dorsal edge of the lower jaw, behind the insertion of the superficial layer. The medial layer is fan-shaped and consists of relatively evenly sized fibres that originate from the lateral side of the parietal and frontal and insert on the medial side of the lower jaw via a broad fascia.

The *m. adductor mandibulae internus* is medial to the *m. adductor mandibulae longus* and consists of two distinct portions. The larger, posterior portion forms a thin, broad sheet of anterodorsally oriented fibres that originate from the taenia marginalis part of the endocranium and the orbital cartilage and

insert directly on the medial side of the lower jaw. A much smaller anterior portion consists of a long and slender bundle of fibres that originate from anteroventral to the eye and insert on the dorsomedial side of the lower jaw via a tendon. Anterior and posterior portions are separated by the optical nerve and associated blood vessels.

The *m. levator quadrati* is a small muscle that consists of short fibres that originate relatively low down the lateral wall of the braincase, just anterior to the quadrate. Its fibres have a vertical orientation and insert dorsally on the pterygoid process of the quadrate.

The *m. pterygoideus* is similar to that of the rhinatrematid larvae but smaller. It originates from the lateral side of the pterygoid (contra Haas, 2001) and inserts on the medial side of the retroarticular process. Ventral to the retroarticular process, its fibres are difficult to distinguish form those of the *m. hyomandibularis*, which is much less developed than in *E. lativittatus*. The *m. hyomandibularis* originates from the ventromedial side of the lower jaw, just underneath the jaw articulation. It is a relatively small muscle that inserts via a short tendon on the lateral side of the distal, flange-like extension of the ceratohyal.

The *m. depressor mandibulae* is a large muscle and has a more complicated architecture than in *E. lativittatus*. It originates from the lateral side of the squamosal, parietal, otic capsule and the dorsal fascia. The fibres that originate from the squamosal and parietal form a thick muscle sheet, whose fibres have a parallel orientation and insert on the dorsal and medial side of the retroarticular process. Its more posterior fibres, which originate from the otic capsule and the dorsal fascia, posterior to the end of the retroarticular process, have an almost vertical orientation and insert along the ventromedial and ventral side of the retroarticular process. Macroscopically, the fibres inserting on the ventral side of the retroarticular process are difficult to separate from those of the *m. pterygoideus* and *m. hyomandibularis*.

The *m. levator hyoideus* is medial to the *m. depressor mandibulae*. The dorsal, fan-shaped portion seen in *E. lativittatus* is absent and only the cranial

portion is present. This muscle is large and fleshy and has very long fibres that originate from the posterior edge of the squamosal, otic capsule and quadrate. The *m. levator hyoideus* is divided into a dorsal and a ventral part by a branch of the facial nerve, although the division between the parts is not as pronounced as in *E. lativittatus*. It inserts broadly on the medial and lateral side of the flange-like extension of the distal ceratohyal.

The *m. intermandibularis* has a similar origin, insertion and fibre orientation as in larval *E. lativittatus*, but extends further posterior, beyond the lower jaw articulation up to about half the length of the retroarticular process.

The *m. interhyoideus* is proportionately larger than in larval rhinatrematids and forms a broad muscular sheet on the lateral and ventral sides of the neck region. Anteriorly, the fibres originate from the lateral side of the ceratohyal, but most of the *m. interhyoideus*, which extends posteriorly to near the gill slits, originates relatively far dorsal form the dorsal fascia. Close to their origin, the fibres of the anterior part originating form the ceratohyal are slightly separted from those of the posterior part originating form the dorsal trunk fascia. The insertion of the *m. interhyoideus* is as in larval rhinatrematids in a mid-ventral fascia. Unlike in larval rhinatrematids, the *m. interhyoideus posterior* of *Ichthyophis* originates from the ventral edge and distal-most tip of the retroarticular process. It covers a much larger area laterally and ventrally and has a more fan-like fibre orientation.

Two larvae at stages 37 and 39 of Dünker et al. (2000) and one metamorphic specimen of *I.* cf. *kohtaoensis* illuminate myological changes during ontogeny. Both larvae have a virtually identical muscle arrangement, except for the extent of the superficial layer of the *m. adductor mandibulae longus*, which covers two thirds of the *m. depressor mandibulae* anteriorly in the stage 37 larva and only one third in the stage 39 larva. Thus the superficial layer of the *m. depressor mandibulae* decreases gradually in size during larval life.

The metamorphic specimen has a greatly reduced superficial layer of the *m. adductor mandibulae longus* and resembles adult rhinatrematids in that the superficial layer is exposed dorsolaterally, although it does not extend onto the

mediodorsal side of the skull. The *m. adductor mandibulae externus* seems absent, other *mm. adductores mandibulae*, the *m. levator quadrati* and the *m. pterygoideus* are comparable to those of larvae. *Mm. hyomandibularis et levator hyoideus* are greatly reduced in extent and number of fibres. The depressor mandibulae is similar to that of larval specimens. *Mm. intermandibularis* , *interhyoideus et interhyoideus posterior* are also similar to those of the larval specimens except for a greater dorsal extent of the *m. interhyoideus posterior* as a result of the stronger dorsal curvature of the retroarticular process.

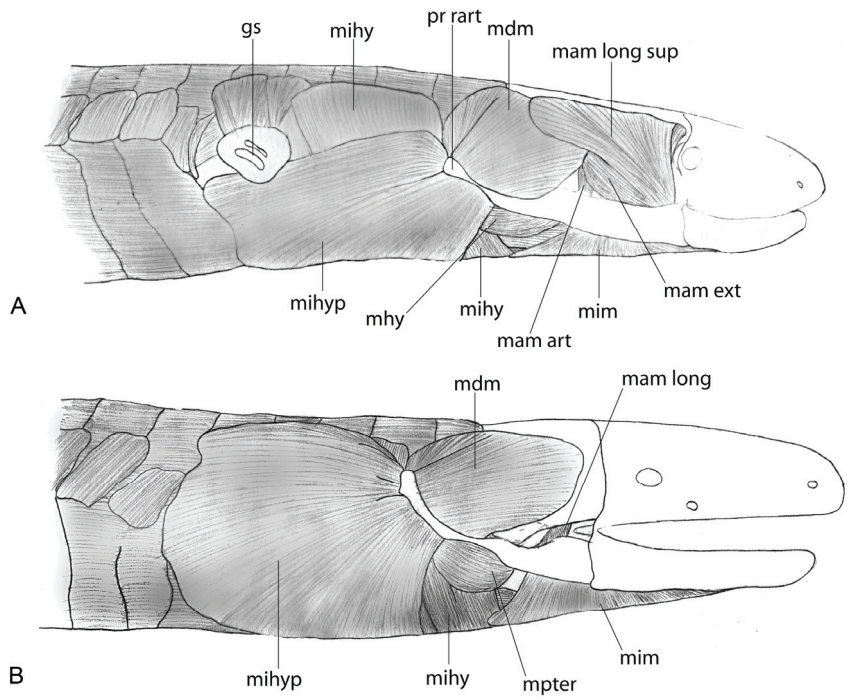

Fig. 15. Superficial musculature of larval (**A**) and adult (**B**) *Ichthyophis bannanicus*. gs, gill slit; mam ext, *m. adductor mandibulae externus*; mam long sup, superficial layer of *m. adductor mandibulae longus*; mam long, *m. adductor mandibulae longus*; mdm, *m. depressor mandibulae*; mihy, *m. interhyoideus*; mihyp, *m. interhyoideus posterior*; mhy, *m. hyomandibularis*; mim, *m. intermandibularis*; mpter, *m. pterygoideus*; pr rart, retroarticular process. Not to scale.

In adult specimens, the primary jaw adductor musculature is confined to the adductor chamber, which is completely covered by bone dorsally and laterally (Fig. 15B). The *m. adductor mandibulae externus* has disappeared. The *m. adductor mandibulae longus* is still the largest primary jaw adductor but much reduced in size. Individual layers are no longer recognizable and the muscle has its origin from the lateral edges of the parietal and frontal and quadrate. It inserts directly dorsally on the lower jaw. The *mm. adductores mandibulae articularis, mandibulae internus et quadrati* have similar proportions and spatial relationships as in the larvae.

The *m. pterygoideus* is larger than in larvae but maintains the same origin and insertion. The *mm. hyomandibularis et levator hyoideus* have disappeared. The *m. depressor mandibulae* is similar in its spatial relationships to that of larvae. Its anterior fibres, however, now have an almost horizontal orientation caused by the dorsal arching of the retroarticular process.

The *m. intermandibularis* has similar fibre orientation, origin and insertion as in larvae. The *m. interhyoideus* is similar in size to that of larvae. In its anterior part, however, the origin of the fibres has partly shifted. Only the anteriormost fibres originate from the ceratohyal, whereas the origin of the more posterior fibres has shifted onto the ventral side of the retroarticular process. The posterior part of the m. interhyoideus originating from the dorsal trunk fascia is simlar to larvae but seems slightly more expanded posteriorly. The *m. interhyoideus posterior* covers a greater area in the adult, especially dorsolaterally, because of the dorsal arching of the retroarticular process. As a result, its insertion has extended from the ventral half of the trunk onto the dorsolateral side, where it inserts in the dorsal fascia.

Uraeotyphlus* cf. *narayani* and *U. oxyurus

The following account applies to larvae of both species investigated here because they are almost identical in their morphology. In the larval specimens, the adductor manibulae musculature is less extensively developed than in rhinatrematid or ichthyophiid larvae (Fig. 16A). There is no distinct *m. adductor*

mandibulae externus. A few fibres have a similar orientation and origin and insertion sites as the *m. adductor mandibulae externus* in rhinatrematid and ichthyophiid larvae but these are not separated from those of the *m. adductor mandibulae longus*. The *m. adductor mandibulae articularis* has its origin, insertion and fibre orientation as in ichthyophiid larvae but is comparatively larger.

The *m. adductor mandibulae longus* is the largest primary jaw adductor in uraeotyphlid larvae also. It is, however, less extensively developed than in rhinatrematid and ichthyophiid larvae and overlaps only a very small part of the *m. depressor mandibulae* anterodorsally. A superficial layer originates from the fascia of the *m. depressor mandibulae*, parietal, frontal, quadrate and the posterior taenia marginalis part of the endocranium and inserts directly on the dorsal side of the lower jaw. The posterior layer is not differentiated from the superficial layer. The medial layer is small and forms a narrow, anteriorly positioned muscle sheet that originates from the lateral margin of the frontal and inserts relatively far posteriorly on the dorsomedial side of the lower jaw via a broad and long tendon.

The *m. adductor mandibulae internus* forms a thin and broad sheet medial to the *m. adductor mandibulae longus*, similar to that of ichthyophiid larvae but more narrow. It consists of two portions, a larger posterior portion originates almost exclusively from the orbital cartilage and inserts on the medial side of the lower jaw via a broad fascia, and a much smaller anterior portion that originates from anteroventrally to the eye and inserts on the same fascia as the posterior portion. As in ichthyophiid larvae, anterior and posterior portions are separated by the optical nerve.

The *m. levator quadrati* is slightly larger in uraeotyphlid larvae than in *E. lativittatus* and ichthyophiid larvae. It also originates more dorsally, resulting in slightly longer fibres that insert dorsally on the pterygoid process of the quadrate.

The *m. pterygoideus* originates from the lateral side of the pterygoid and inserts on the medial side of the retroarticular process. The *m. hyomandibularis* is

larger and more clearly separated from the *m. pterygoideus* than in ichthyophiid larvae. It originates from the ventral side of the lower jaw and runs along the ventrolateral side of the retroarticular process. Its insertion is on the lateral side of the distal ceratohyal, anterior to the insertion of the m. adductor hyoideus.

The *m. depressor mandibulae* is a large muscle and similar in architecture to that of ichthyophiid larvae. It originates from the lateral side of the squamosal, parietal, otic capsule and the dorsal fascia, and inserts on the dorsal and ventral side of the retroarticular process. The fibres that insert on the ventral side of the retroarticular process are completely separated from those of the *m. hyomandibularis*.

The *m. levator hyoideus* is similar to that of ichthyophiid larvae, but much smaller. It originates from the posterior edge of the squamosal via a broad fascia and inserts on the dorsolateral, dorsal and dorsomedial side of the distal ceratohyal. Separate dorsal and ventral parts are not differentiated.

The *m. intermandibularis* has a similar origin and insertion to that of *Ichthyophis*. It does not extend to the anterior-most area between the lower jaw ramis, where it leaves a small area uncovered. Posteriorly, it extends to just beyond the jaw articulation. Its fibres have a fan like orientation and meet along the ventral midline, with divergences at its anterior and posterior end.

The *m. interhyoideus* resembles the more posterior portion of that muscle in ichthyophiid larvae and has its sole origin from the dorsal fascia, with no fibres originating from the ceratohyal. The more anterior fibres, however, are relatively long and obliquely oriented and cover a similar area as those covered by the anterior portion of the *m. interhyoideus* originating form the ceratohyal in larval *Ichthyophis*. The *m. interhyoideus posterior*, is similar in size to that of ichthyophiid larve and also originates from the ventral edge and distal-most tip of the retroarticular process. It is, however, split into a smaller, dorsolateral slip and a larger, ventrolateral slip and between both slips, towards their posterior ends, is the spiracular opening.

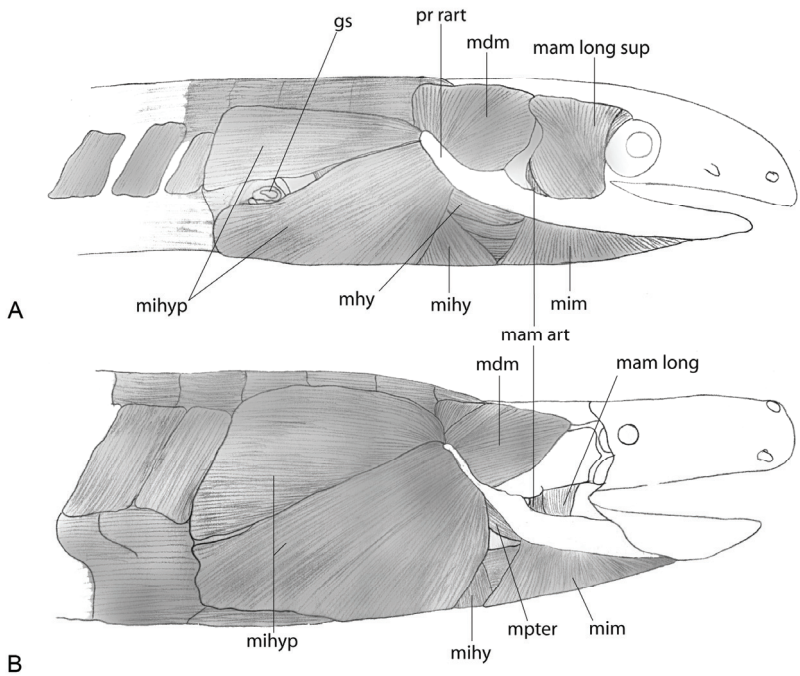

Fig. 16. Superficial musculature of larval (**A**) and adult (**B**) *Uraeotyphlus* cf. *narayani*. gs, gill slit; mam art, *m. adductor mandibulae articularis*; mam long sup, superficial layer of *m. adductor mandibulae longus*; mam long, *m. adductor mandibulae longus*; mdm, *m. depressor mandibulae*; mihy, *m. interhyoideus*; mihyp, *m. interhyoideus posterior*; mhy, *m. hyomandibularis*; mim, *m. intermandibularis*; mpter, *m. pterygoideus*; pr rart, retroarticular process. Not to scale.

Superficially, the musculature of adult *Uraeotyphlus* cf. *narayani* (Fig. 16B) resembles that of the adult *Ichthyophis* investigated here. The primary jaw adductor musculature is confined to the adductor chamber. The *m. adductor mandibulae articularis* is a relatively thick muscle with similar spatial relationships to those of larvae. The *m. adductor mandibulae longus* is narrower than in larvae and has no distinguishable layers. Its dorsoposterior part is visible through a window-like fascia between the parietal, squamosal and quadrate, which is covered by the *m. depressor mandibulae*. The *m. adductor mandibulae internus* is similar to that of larvae but reaches a little further anterodorsally and its anteriormost fibres have their origin from the ventrolateral margin of the

frontal. The *m. levator quadrati* has similar spatial relationships to those of larvae.

The *m. pterygoideus* is larger than in larvae and the fibres ventral and lateral to the retroarticular process have an almost vertical orientation. The *m. hyomandibularis* has disappeared.

The *m. depressor mandibulae* is proportionately a little smaller than in larvae and has a roughly triangular shape in lateral view. It has almost straight, ventrolateral and dorsomedial margins that converge to form a blunt anterior tip.

The *mm. intermandibularis et interhyoideus* are similar in to that of larvae. The *m. interhyoideus posterior* is still split into a dorsal and ventral slip as in larvae, but both slips now tightly abut each other except for their posterior-most parts. The dorsal slip has expanded dorsally and both slips are now more equally sized, with the ventral still being slightly larger.

Praslinia cooperi

In the larva (Fig. 17A), the adductor mandibulae musculature is superficially similar to that of uraeotyphlids. The *m. adductor mandibulae externus* is relatively indistinct; its fibres are almost horizontal, originate directly from the dorsal side of the lower jaw and insert on the anterior side of the quadrate. The *m. adductor mandibulae articularis* is comparatively smaller and has shorter fibres than in the previously described taxa, but maintains a similar origin, insertion and fibre orientation.

The *m. adductor mandibulae longus* does not overlap the depressor mandibulae. It originates from the parietal, frontal and quadrate and has a relatively complicated insertion on the lower jaw and lip fold of the corner of the mouth, with some of the fibres inserting directly on the dorsal and dorsomedial side of the lower jaw, while others insert via a short tendon in the connective tissue of the lip fold. Furthermore, the *m. adductor mandibulae longus* has a complex fibre arrangement, some of which is reminiscent of the superficial, posterior and medial layers seen in this muscle in larvae of *Epicrionops lativittatus* and ichthyophiids. These however, can not be separated

macroscopically and it is unlikely that they constitute separate layers in *Praslinia cooperi*.

The *m. adductor mandibulae internus* is a thin, sheet-like muscle as in the other taxa. It originates mainly from the taenia marginalis part of the os basale, with a smaller, anterior slip originating from anteroventral of the eye. Both slips insert on the medial side of the lower jaw. The smaller, anterior portion is less widely separated from the main slip than in ichthyophiid or uraeotyphlid larvae and seems to originate further posteriorly than in these taxa and rhinatrematids. As in ichthyophiid and uraeotyphlid larvae, anterior and posterior portions are separated by the optical nerve.

The *m. levator quadrati* is very small and forms a narrow muscle with short fibres, which originate from the lateral side of the braincase and insert dorsally on the pterygoid process of the quadrate.

The *m. pterygoideus* originates from the lateral side of the pterygoid and inserts on the medial side of the retroarticular process.

The *m. hyomandibularis* is a large muscle with long fibres that has a broad origin from the ventral side of the lower jaw, from far anterior of the jaw articulation, extending posteriorly to about half way along the length of the retroarticular process. Its more dorsal fibres insert directly on the lateral side of the distal third of the ceratohyal. Most of its ventral fibres, however, insert into a fascia that attaches mainly to the posterior edge of the ceratohyal, but which also forms an attachment site for the dorsal-most fibres of the m. subarcualis rectus I.

The *m. depressor mandibulae* is large. It originates from the lateral side of the squamosal, parietal, otic capsule and the dorsal fascia. Its insertion is confined to the dorsal side of the retroarticular process, as in rhinatrematid larvae.

The *m. levator hyoideus* is similar to that of rhinatrematid larvae. Two distinct portions are present, a small, fan-shaped dorsal portion that originates from the dorsal fascia and inserts on the distal-most tip of the ceratohyal, and a second, much larger cranial portion that originates from the posterior edge of the squamosal, otic capsule and quadrate and inserts on the dorsolateral, dorsal and

dorsomedial side of the distal ceratohyal. Distinct dorsal and ventral parts are not differentiated.

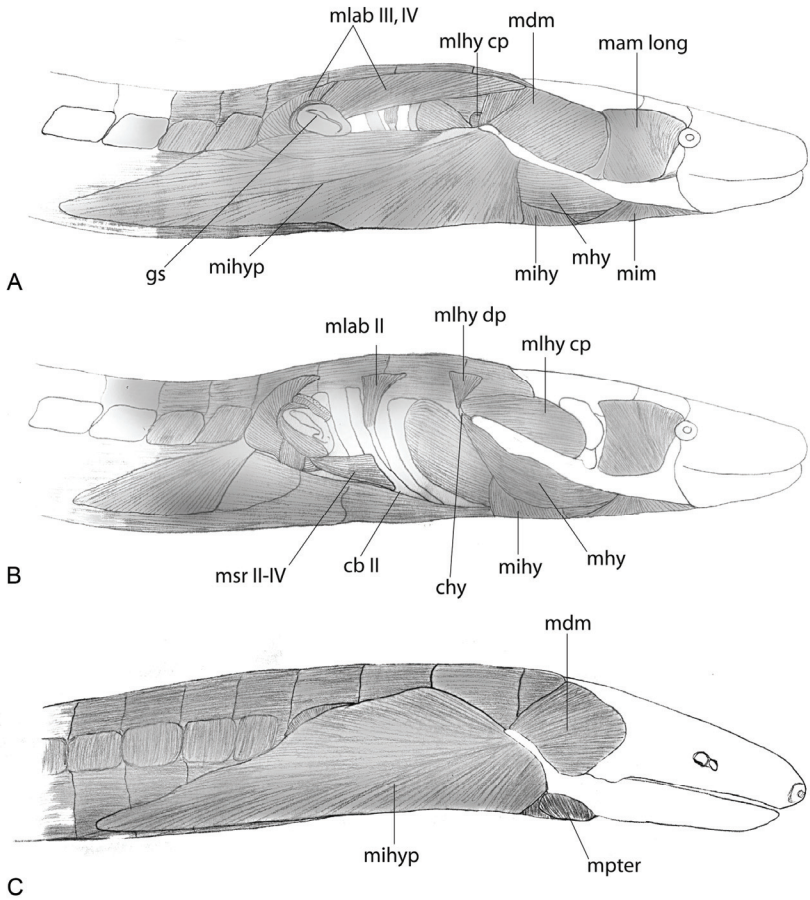

Fig. 17. Musculature of larval and adult *Praslinia cooperi*. **A** superficial musculature of larva. **B** Larva, with *m. depressor mandibulae* and *m. interhyoideus posterior* removed. **C** Superficial musculature of adult. cb II, ceratobranchial II; chy, ceratohyal; gs, gill slit; mam long, *m. adductor mandibulae longus*; mdm, *m. depressor mandibulae*; mihy, *m. interhyoideus*; mihyp, *m. interhyoideus posterior*; mlhy cp, cranial portion of *m. levator hyoideus*; mlhy dp, dorsal portion of *m. levator hyoideus*; mhy, *m. hyomandibularis*; mim, *m. intermandibularis*; mlab II, *m. levator arcus branchialis* II; mlab III, IV, *m. levator arcus branchialis* III, IV; mpter, *m. pterygoideus*; msr II-IV, *m. subarcualis rectus* II-IV. Not to scale.

The *m. intermandibularis* has a similar origin and insertion as in the aforementioned taxa but is much smaller. Its fibres have a distinctly fan-shaped orientation, do not meet in the ventral midline and leave a relatively large area uncovered anteriorly.

The posterior half of the *m. interhyoideus* is covered by the *m. interhyoideus posterior* and the fibres of both muscles are not very well separated near their mid-ventral insertion. The *m. interhyoideus* originates from the lateroventral side of the ceratohyal, relatively far medial. The *m. interhyoideus posterior* originates from the ventral edge of the retroarticular process and is much larger than in aforementioned taxa. It extends along the ventrolateral side of the body and reaches the eighth dorsal trunk myomere posteriorly. Its anterior fibres converge towards the ventral mid-line, whereas its posterior fibres have a more oblique, posteroventral orientation. Most of its fibres originate directly from the retroarticular process, except for the dorsal-most fibres, which originate from the retroarticular process via a fascia. These dorsal-most fibres have a parallel, horizontal orientation, which sets them apart from the other fibres of the *m. interhyoideus posterior*.

Only the superficial musculature was examined in an adult *Praslinia cooperi* (Fig. 17C). All primary adductor muscles are covered by bone. The *m. pterygoideus* is relatively prominent in lateral and ventral view and proportionately larger than in larvae, but maintains the same origin and insertion. The *mm. hyomandibularis et levator hyoideus* have disappeared. The *m. depressor mandibulae* has similar fibre orientation, origin and insertion as in conspecific larvae, as does the *m. intermandibularis*. The *mm. interhyoideus et posterior* are similar in their arrangement to the larva and sistincly separate. The m. interhyoideus posterior has slightly expanded dorsally.

Sylvacaecilia grandisonae

In the larval specimen (Fig. 18A), the size of the superficial adductor mandibulae musculature is similar to that of uraeotyphlid or *Praslinia cooperi* larvae. A distinct *m. adductor mandibulae externus* is absent. A few fibres have a similar

orientation and origin as the *m. adductor mandibulae externus* in rhintrematid and ichthyophiid larvae, but insert in the labial fold. The *m. adductor mandibulae articularis* is rather small, originates from the anterior side of the quadrate and inserts on the lower jaw just in front of the jaw articulation.

The *m. adductor mandibulae longus* is the largest primary jaw adductor. It is similar in shape to that of *Praslinia cooperi* larvae and does not overlap the *m. depressor mandibulae* but tightly abuts it. It originates from the fascia of the *m. depressor mandibulae*, parietal, frontal, quadrate. The insertion on the lower jaw is complex, with some fibres having a direct dorsal insertion whereas other insert on the dorsal and dorsomedial side of the lower jaw via a broad fascia. As in *Praslinia cooperi* larvae, the fibre architecture is complex, but separate layers as in rhinatrematid, ichthyophiid and uraeotyphlid larvae are macroscopically not distinguishable.

The *m. adductor mandibulae internus* forms a thin and broad sheet, similar to the condition in ichthyophiid larvae. As in ichthyophiid larvae, it consists of two portions, a larger posterior portion that originates from the taenia marginalis part of the endocranium and the orbital cartilage, and a much smaller anterior portion that originates further anterior, ventral to the eye. Both insert on the medial side of the lower jaw via a common, broad fascia.

The *m. levator quadrati* consists of rather short fibres but is relatively broad. It originates from the lateral wall of the braincase and inserts dorsally on the pterygoid process of the quadrate.

The *m. pterygoideus* originates from the lateral side of the pterygoid process of the quadrate and inserts on the medial side of the retroarticular process, just posteriorly of the jaw articulation.

As in *Praslina cooperi* larvae, the *m. hyomandibularis* is larger and more clearly separated from the *m. pterygoideus* than in ichthyophiid larvae. It has a broad origin from the ventral side of the lower jaw of about half the length of the retroarticular process from the jaw articulation. Its fibres are much shorter than in *Praslinia cooperi* larvae and it also has a broader insertion on the lateral side of the ceratohyal.

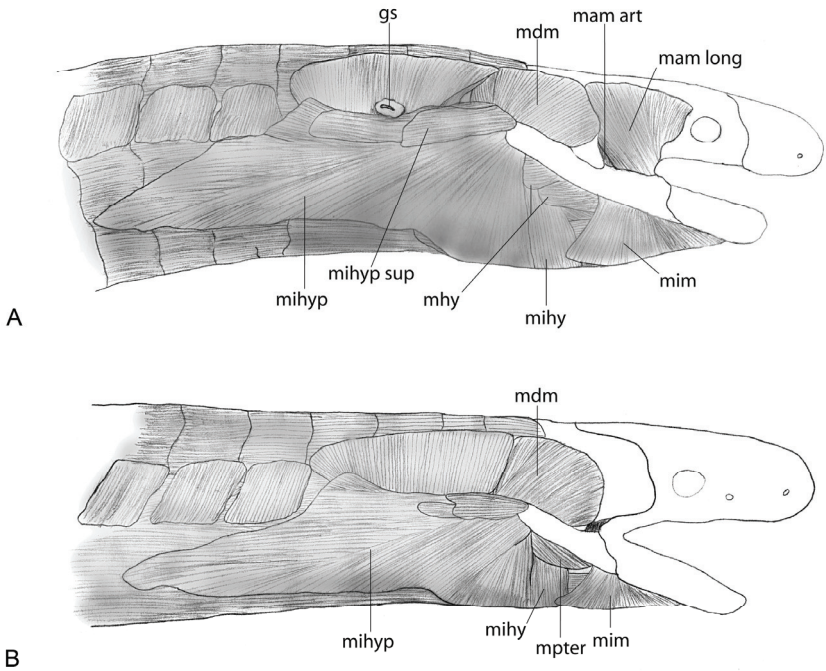

Fig. 18. Superficial musculature of larval (**A**) and adult (**B**) *Sylvacaecilia grandisonae*. gs, gill slit; mam art, *m. adductor mandibulae articularis*; mam long, *m. adductor mandibulae longus*; mdm, *m. depressor mandibulae*; mihy, *m. interhyoideus*; mihyp, *m. interhyoideus posterior*; mihyp sup, superficial slip of *m. interhyoideus posterior*; mhy, *m. hyomandibularis*; mim, *m. intermandibularis*; mpter, *m. pterygoideus*. Not to scale.

The *m. depressor mandibulae* is a large muscle and similar in architecture to that of ichthyophiid and uraeotyphlid larvae. It originates from the lateral side of the squamosal, parietal, otic capsule and the dorsal fascia and inserts on the dorsal and ventral side of the retroarticular process. The fibres that insert on the ventral side of the retroarticular process are completely separated from those of the *m. hyomandibularis*.

The *m. levator hyoideus* is much smaller than in *Praslinia cooperi* or non-caeciliid larvae. It originates from the posterior side of the ventral half of the quadrate and inserts on the dorsolateral, dorsal and dorsomedial side of the distal ceratohyal. Separate dorsal and ventral portions are not differentiated. A dorsal,

fan-shaped part is absent. Origin, insertion, size and fibre orientation of the *m. intermandibularis* are similar to those of larval *Praslinia cooperi*.

The *m. interhyoideus* originates from the ventral edge of the ceratohyal and inserts in a mid-ventral fascia. Its parallel fibres have an anteroventral orientation, as in rhinatrematid and ichthyophiid larvae. The *m. interhyoideus posterior* has a similar shape and extent to that in larval *Praslinia cooperi*. Most of its fibres originate from the ventral edge of the retroarticular process, but some of its dorsal fibres originate from the anterior edge of the distal part of the retroarticular process. These form a short superficial slip and a medial slip, twice as long as the superficial slip, and are characterized by horizontally oriented, parallel fibres.

Adult *Sylvacaecilia grandisonae* (Fig. 18B) have a similar arrangement of the superficial musculature to adult *Praslinia cooperi*. All primary adductor muscles are covered by bone. The *m. pterygoideus* is somewhat larger than in larvae but maintains the same origin and insertion. The *mm. hyomandibularis et levator hyoideus* have disappeared. The *m. depressor mandibulae* has similar fibre orientation, origin and insertion as in conspecific larvae, as does the *m. intermandibularis*. The *mm. interhyoideus et posterior* are not very distinct close to their insertion on the midventral fascia where they seem to be partly fused. The anterior fibres have a relatively broad origin from the ceratohyal but at least some fibres of the *m. interhyoideus* have seemingly shifted their origin to the ventral side of the retroarticular process. The dorsal, superficial slips of the *m. interhyoideus posterior* are still present but much reduced in size.

Grandisonia* cf. *larvata* and *G. sechellensis

Larval specimens of both species are similar in their myology. The following account applies to both species, but existing differences are highlighted. Size and shape of the superficial adductor mandibulae musculature is similar to that of uraeotyphlid or other caeciliid larvae. A distinct *m. adductor mandibulae externus* is absent (Fig. 19A). The *m. adductor mandibulae articularis* originates from the anterior side of the quadrate and inserts on the lower jaw just in front of the jaw

articulation. It is relatively smaller in *Grandisonia* cf. *larvata* than in *G. sechellensis*.

The *m. adductor mandibulae longus* is the largest primary jaw adductor and does not overlap the *m. depressor mandibulae*. It originates from the fascia of the depressor mandibulae, parietal, frontal, and quadrate. Insertion on the lower jaw and the fibre architecture of this muscle are complex, as in the other caeciliid larvae investigated. In the small *G. sechellensis* larva investigated, we were able to dissect layers corresponding to the superficial, posterior and medial layers seen in larvae of *Epicrionops lativittatus* and ichthyophiids. However, these layers could not be detected macroscopically in the larger *G. sechellensis* larva and the *G.* cf. *larvata* larva investigated here.

The *m. adductor mandibulae internus* is essentially similar to that of other caecilian larvae, consisting of a large posterior slip and a much smaller anterior slip, which respectively originate from the taenia marginalis part of the os basale and orbital cartilage, and a position anteroventral to the eye, and insert on the medial side of the lower jaw via a broad fascia.

The *m. levator quadrati* is a small muscle that originates from the lateral wall of the braincase and inserts dorsally on the pterygoid process of the quadrate. Ventral to its insertion is the origin of the *m. pterygoideus*, which itself inserts on the medial side of the anterior half of the retroarticular process.

The *m. hyomandibularis* is relatively small but well separated from the *m. pterygoideus*. Its fibres have an almost horizontal orientation, originate from the ventral side of the lower jaw just underneath the jaw articulation, and insert on the lateral side of the distal ceratohyal. The insertion is more medial, further away from the distal tip of the ceratohyal, than in all other caecilian larvae. The *m. depressor mandibulae* is similar in size, origin and insertion to that of the other caeciliid larvae. A *m. levator hyoideus* is absent in larvae and adults.

Origin, insertion, size and fibre orientation of the *m. intermandibularis* are similar to those of the other caeciliid larvae, and the *m. interhyoideus* resembles that of larval *Sylvacaecilia grandisonae*. The shape of the *m. interhyoideus posterior* is similar to that of the other caeciliid larvae but its extent

is different in larval *Grandisonia* cf. *larvata* and *G. sechellensis*. The muscle originates from the ventral edge of the retroarticular process in both species. In *G. sechellensis*, the dorsal fibres have a parallel arrangement and are horizontally oriented, as in larval *P. cooperi* and *S. grandisonae*, whereas they have a simple, oblique orientation in *G.* cf. *larvata*. Both species differ further in the posterior extension of the *m. interhyoideus posterior*, which reaches backwards to the posterior end of the sixth dorsal trunk myomere in *G.* cf. *larvata* and to the posterior end of the seventh dorsal trunk myomere and slightly beyond in *G. sechellensis*.

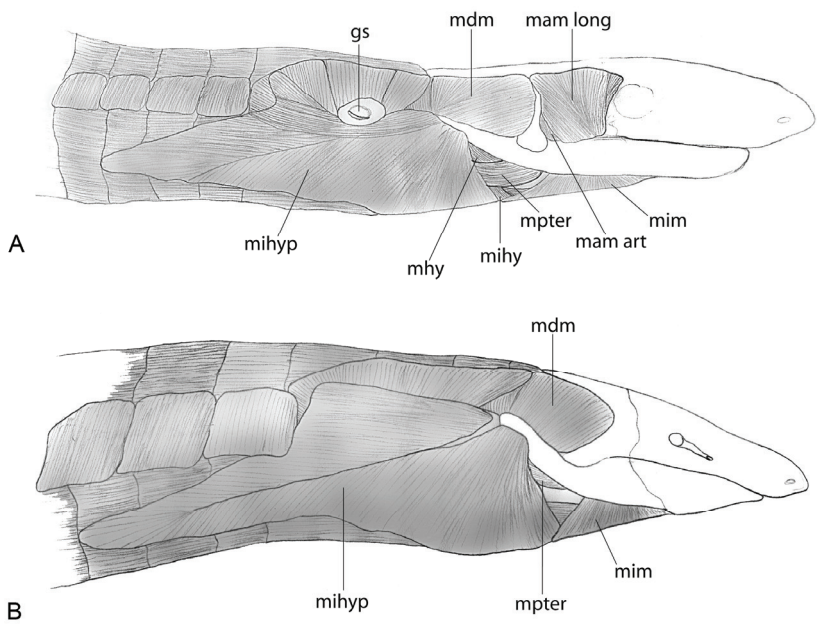

Fig. 19. Superficial musculature of larval (**A**) and adult (**B**) *Grandisonia sechellensis*. gs, gill slit; mam art, *m. adductor mandibulae articularis*; mam long, *m. adductor mandibulae longus*; mdm, *m. depressor mandibulae*; mihy, *m. interhyoideus*; mihyp, *m. interhyoideus posterior*; mhy, *m. hyomandibularis*; mim, *m. intermandibularis*; mpter, *m. pterygoideus*. Not to scale.

Adult *G. sechellensis* (Fig. 19B) have a very similar muscle arrangement to the other adult caeciliids investigated here. The primary jaw adductor musculature is confined to a closed adductor chamber. The *m. adductor*

mandibulae articularis is comparatively small and consists of few, short fibres that maintain a similar origin and insertion as in larvae. The *m. adductor mandibulae longus* is still the largest of the primary adductors, but proportionately much smaller than in larvae. It comprises a single muscle, with no macroscopically distinguishable layers, that originates from the lateral edge of the parietal and inserts on the dorsomedial side of the lower jaw. The *m. adductor mandibulae internus* is also proportionately smaller. It is broader than in the larva, has much shorter fibres, originates from the dorsolateral side of the os basale and inserts on the medial side of the lower jaw via a broad and long fascia. An anterior slip originates from beneath the orbitonasal orifice and inserts in the same fascia as the main slip.

The *mm. levator quadrati et pterygoideus* have similar spatial relationships, origin and insertion as in larvae and the *m. hyomandibularis* has disappeared. The *m. depressor mandibulae* is similar to that of the other adult caeciliids investigated here. A *m. levator hyoideus* is absent.

The *m. intermandibularis* is similar in extent, origin and insertion as in larvae. The fibres of the *mm. interhyoideus et interhyoideus posterior* seem to be partially fused and are difficult to distinguish towards their insertion on the ventromedial fascia. The anteriormost fibres of the *m. interhyoideus* have a narrow origin on the ceratohyal, whereas the remainder originate from the retroarticular process. It is a large, pinnate muscle, with a strong median fascia that originates from the distal tip of the retroarticular process and runs posteriorly for almost the entire length of the muscle.

DISCUSSION

Skull morphology

The skulls of several of the species investigated here have been previously described by other authors and only larger differences will be noted, where these exist. Nussbaum (1977) figured and described the skull of adult *Epicrionops petersi* and also provided a comparative description of the skull of adult *Rhinatrema bivittatum*. Reiss (1996) provided a description of the larval skull and

adult skull of *E. bicolor* and *E. petersi* and focussed particularly on the morphology of the palate and its remodeling during metamorphosis. Wake (2003) provided figures of the skull of larval and adult *E. bicolor* together with a short characterisation and Wake (1989) made some brief comments on the morphology of the larval skull. All these descriptions are consistent with our results for *E. lativittatus* and skull morphology seems to be fairly similar among rhinatrematids, at least in different *Epicrionops* species. Nussbaum (1977), however, considered the rhinatrematid posterior pterygoid to be the pterygoid process of the quadrate. Reiss (1996) investigated palatal metamorphosis in *Epicrionops* and inferred that the single pterygoid of larvae divides into an anterior and posterior part during metamorphosis. Until a detailed ontogenetic series becomes available to study this phenomenon in more detail, we agree with Reiss (1996) that the different morphology of the larval pterygoid as compared to the adult is best explained by a split of the single larval into the two adult pterygoids during metamorphosis.

Larval and adult skulls of various species of *Ichthyophis* have been described by several authors (e.g. Wiedersheim, 1879; Sarasin & Sarasin, 1887-1890; Peter, 1898; de Beer, 1937; Ramaswami, 1947; Visser, 1963; Taylor, 1969; Wake, 2003). All these studies and our own investigations show that different species of *Ichthyophis* are very similar in both their larval and their adult skull morphology. Most taxonomic and ontogenetic variation occurs in the position of the tentacular opening and the degree as to which the tentacular canal is roofed and separated from the orbit, and the size of the slight temporal gap sometimes present in ichthyophiids. Variation particularly in larval skulls seems to be size related; skulls of mature larvae of *I. bannanicus* seem to be proportionately broader than in mature larvae of *I.* cf. *kohtaoensis*, which apparently metamorphoses at smaller sizes than *I. bannanicus*. Further variation also exists in the number, size and shape of the palatal cartilage of larvae, which can be single or paired, small and spherical or larger and more elongated.

Peters (1881) and Parker (1927) provided brief and relatively general descriptions of the skull of adult *Uraeotyphlus oxyurus*, and *U. oxyurus* and *U.*

malabaricus, respectively, and Taylor (1969) presented a photograph and brief description of an adult *U. oxyurus* skull. Ramaswami (1941) and Nussbaum (1979) provided detailed descriptions of the skull of adult *Uraeotyphlus narayani* and Wake (2003) figured the adult skull of *U. narayani* and provided a short general description. The larval skull has not been described before, but its general similarity with the larval skull of ichthyophiids, apart from the shape of the squamosal and the palatal cartilage, mirrors the similarity between adult skulls of ichthyophiids and uraeotyphlids.

Parker (1941) provided some limited information on the morphology of the adult skull of *Praslinia cooperi*, stating that a well developed ectopterygoid is present and that the gap between squamosal and parietal is similar in extent to that of other Seychellean caeciliids, contrary to Werner (1931) who considered squamosal and parietal to be separated in *Praslinia* but in contact in *Hypogeophis* and *Grandisonia*. Wake (2003) provided a short characterization of the adult skull of *Praslinia cooperi*, summarizing the terse account of Parker (1941), and concluded that it is similar to that of the other Seychellean genera (*Grandisonia* and *Hypogeophis*). This conclusion is not supported by our observations. The skull of *P. cooperi* is very different from the narrow and pointed skulls of *Hypogeophis rostratus* and *Grandisonia* spp. It is more similar to large specimens of *Dermophis*, *Gymnopis* or *Siphonops*, but shows a more pronounced dorsoventral compression than in these genera. Of particular interst is the presence of a relatively large pterygoid-like element in the adult, which is absent in the larva. The larva, however, has a conspicuous anterolateral process on the pterygoid process of the quadrate in a similar position to the pterygoid-like element of the adult. A larval *Sylvacaecilia grandisonae* shows some contralateral variation of this character in that it possesses a free pterygoid-like element on one side and a process of the pterygoid process similar to that seen in larval *P. cooperi*. It might be that the pterygoid-like element only appears later during ontogeny and is therefore not present in the larva. Alternatively, the pterygoid-like element might be somewhat labile in that it can either fuse to the pterygoid process or be separate. In that case, the element would have already

been fused to the pterygoid process in the larva. The consistent reports of a free pterygoid-like element in adult *P. cooperi* (Parker, 1941; this study), however, make this less likely. Wilkinson & Nussbaum (1992) inferred the splitting-off of a small pterygoidal element (pseudoectopterygoid of Wilkinson & Nussbaum, 1992) from the pterygoid process of the quadrate during development in siphonoform caeciliids (*Siphonops* and related taxa, see Wilkinson & Nussbaum, 1992), and a similar mechanism might be responsible for the observed pattern in *P. cooperi* (and possibly *S. grandisonae*).

Wake (1987), in her description of the genus *Sylvacaecilia*, figured the skull of *S. grandisonae* and provided a description of its main characteristics. The specimen on which her description is based (BMNH 1976.1131) was variously characterized as a late larva or juvenile (Wake, 1987), but is better considered a juvenile because neither its skull nor hyobranchial skeleton show any larval characteristics (see also Reiss, 1996). The tentacular canal, however, is not completely closed in this specimen but continuous with the orbit. During ontogeny, the tentacle migrates form a position close to the eye to about half way between nostril and eye in adult *S. grandisonae* (Largen *et al.*, 1972), and Taylor (1970) found the tentacular canal discontinuous with the orbit in a specimen of 259 mm total length. This indicates that the tentacular groove is likely a juvenile characteristic, forming a fully roofed tenatcular canal in adults. Of more profound disagreement between Wake (1987) and the present study is the presence of an ectopterygoid or pterygoid-like element in both the larval and juvenile *S. grandisonae*. Wake (1987) examined the same juvenile specimen also examined in this study, and reported the absence of an ectopterygoid, which she considered to be a diagnostic character of the genus *Sylvacaecilia*, and the ectopterygoid is ommited in her drawing of the skull of BMNH 1976.1131. A more detailed drawing of the same specimen is presented in Wake (2003) but does also not indicate the ectopterygoid, and the absence of the ectopterygoid is again mentioned in the brief description of the characteristics of the skull of *Sylvacaecilia*. However, an ectopterygoid or pterygoid-like element is clearly present in both larvae and metamorphosed *S. grandisonia* (cf. Fig. 9 and Fig. 10).

Parker (1941) provided a partial description and a figure in dorsal view of the adult skull of *Grandisonia sechellensis*. Taylor (1969) provided photographs of the adult skull of *G. alternans* and *G. sechellensis* in dorsal, ventral and lateral view, and a short description. Straub (1985, 1986) studied the cranial anatomy of all *Grandisonia* spp. (except *G. brevis*) based on reconstructions of serial sections and provided detailed descriptions of the morphology of the head in all species of *Grandisonia* except *G. brevis*. Somewhat confusing is the presence of an ectopterygoid or pterygoid-like element among species of the genus *Grandisonia*. According to Straub (1985, 1986), the element is absent in *G. sechellensis*. This is confirmed here, no pterygoidal element is present in the larval or adult specimens examined. However, as noted by Straub (1985), Taylor (1969) presented a heavily retouched photograph of the skull of an adult *G. sechellensis*, which seems to show a pterygoidal element. Straub (1985) suggested a misidentification of Taylor's specimen as the most likely reason for the observed discrepancy. A taxonomic misidentification might also explain the absence of a pterygoidal element in the larval *G.* cf. *larvata* examined here, a species which possesses a pterygoidal element according to Straub (1985). Another possible explanation is ontogentic variation regarding the presence of this element in larval and adult *G. larvata*. Among the other species possessing a pterygoidal element according to Straub (1985), is *G. diminutiva*, which, according to Wilkinson & Nussbaum (2006), represents a junior synonym of *G. sechellensis*, a species which does not possess a pterygoidal element. Further examination of more material of undisputed identification will be necessary to clarify this issue.

Hyobranchial skeleton

Wake (1989) described the morphology of the larval hyobranchial skeleton and its metamorphosis in *Epicrionops bicolor* and *E. petersi* and stated that the larval hyobranchial skeleton consist of the paired ceratohyal and four pairs of ceratobranchials plus a series of unpaired medial elements consisting of the basihyal and three basibranchials. This contrasts with the figure of the larval

hyobranchial skeleton of *E. bicolor* in Wake (1989) that shows four instead of three basibranchials and another figure of a larval hyobranchial skeleton of the same species in Wake (2003), which shows only two basibranchials, similar to *E. lativittatus* as described in the present paper. While a *lapsus calami* seems to be the most likely explanation for the discrepancy in basibranchial number in *E. bicolor* between the description and the figure in Wake (1989), it seems unlikely that the same reason is responsible for the discrepancies between Wake (1989) and Wake (2003). A more plausible explanation seems to be interspecific variation in the number of basibranchial elements or a progressive reduction of basibranchials during ontogeny. The adult hyobranchial skeleton of *Epicrionops* has been described by Nussbaum (1977) and Wake (1989, 2003) and these descriptions are similar to the observations made here of the adult hyobranchial skeleton in *E. lativittatus*.

The larval and adult hyobranchial skeleton of *Ichthyopis* have been described several times by various authors (e.g. Sarasin & Sarasin, 1887-1890; Visser, 1963; Nussbaum, 1977; 1979; Wake, 2003) and are in line with the results of the present study. The fusion of the ceratohyal and the ceratobranchial in the larval *I. bannanicus* seems to indicate the onset of the metamorphic remodelling of the hyobranchial skeleton, although this specimen does not show other signs of the onset of metamorphosis.

Ramaswami (1941) and Wake (2003) described the adult hyobranchial skeleton of *U. narayani*. The larval hyobranchial skeleton is very similar to that of larval *Ichthyophis*, except for the differently shaped basihyal.

The adult hyobranchial skeleton of *Praslinia cooperi* has the typical morphology seen in other caeciliid and most typhlonectid species (Nussbaum, 1977; Wilkinson & Nussbaum, 1997; Wake, 2003). The larval hyobranchial skeleton, however, is similar to that of larval ichthyophiid or uraeotyphlid caecilians in that it retains a separate basihyal, basibranchial and ceratobranchials III and IV. It is further remarkable in that the ceratohyal and ceratobranchials as well as the basihyal stain positive for Alizarin red. Inspection at high

magnification shows that the Alizarin stained areas are composed of calcified cartilage, rather than bone.

Wake (1987, 2003) figured and described the hyobranchial skeleton of a juvenile of *Sylvacaecilia grandisonae*. As in other caeciliids, ceratohyal, basihyal and ceratobranchial I are fused into a single structure. However, fusion of ceratobranchial III and IV is not complete and ceratobranchial IV forms a broad, disc-like medial process, while the distal tip of ceratobranchial III is still distinguishable. The larval hyobranchial skeleton also stains positive for Alizarin red in large parts and, as in *Praslinia cooperi*, these areas are composed of calcified cartilage and not bone in contrast to larval rhinatrematids.

The adult hyobranchial skeleton of *Grandisonia sechellensis* has a morphology typical of other caeciliid species (see above and Fig. 13). Straub (1985) illustrated hyobranchial skeletons of metamorphosed specimens of *G. larvata* and *G. sechellensis*. Both are similar but show some variation in proportions, i.e. the distal ceratobranchial I is figured to be broader in *G. larvata*, while *G. sechellensis* has a broader ceratobranchial III+IV. These features are ontogenetically variable and might not be specifically distinct and our adult *G. sechellensis* specimen has a similar hyobranchial morphology to that described by Straub (1985). The larval hyobranchial skeleton is characterized by separate basihyal and basibranchial, whereas the ceratobranchial IV is already fused with the ceratobranchial III along its medial end, but retains a long, filiform lateral part that is separate from the similarly slender lateral part of ceratobranchial III. The lateral end of ceratobranchial III is further coiled to support the gill aperture. This is reminiscent of the shape of the embryonic hyobranchial skeleton of *Hypogeophis rostratus* (Müller, 2006; see Chapter 2).

Of particular interest is the mineralized hyobranchial skeleton of larval *Praslinia cooperi* and *Sylvacaecilia grandisonae*. The only other caecilian larvae known to have a mineralzed hyobranchial skeleton are those of the rhinatrematid *Epicrionops* (Wake, 1989). These, however, are composed of true bone, as opposed to the calcified cartilage seen in *P. cooperi* and *S. grandisonae*, and therefore probably not directly homologous. Wake described the unique

metamorphosis of the larval hyobranchial skeleton of *E. bicolor* and *E. petersi*, in which the bony elements are completely resorbed upon metamorphosis and replaced by cartilaginous elements that form *de novo* at the same time. It would be interesting to investigate whether the mineralized cartilages of *P. cooperi* and *S. grandisonae* are replaced in a similar way during metamorphosis or whether a simple decalcification takes place, and future studies should address this question.

Larval morphology and metamorphosis in caecilians

It seems apparent that, despite several obvious differences, larval caecilians share a very similar general morphology that is different from that of adult caecilians. All caecilian larvae are characterized by a gymnokrotaphic skull with a completely open temporal region. Correspondingly, the shape of the squamosal is different in larvae and adults and some variation exists in the investigated taxa. Rhinatrematid, ichthyophiid and *Praslinia cooperi* larvae are characterized by a sickle-shaped squamosal that is attached to the lateral side of the quadrate and extends onto the dorsolateral side of the skull via a dorsoposteriorly directed process (see Fig. 2, 3, 7). Upon metamorphosis, the squamosal grows anteriorly, while the maxilla, which is short in larvae (see Reiss, 1996, 2002), extends posteriorly to cover the cheek region. Adult rhinatrematids retain the dorsoposteriorly direct process whereas it is resorbed in all other species. Other changes at metamorphosis include the fusion of the parasphenoid with the posterior neurocranium and otic capsule to form the os basale, and the fusion of the maxilla with the palatine into the maxillopalatine. However, in *Epicrionops*, fusion of the parasphenoid occurs prior to metamorphosis and the os basale is already formed in early larvae (see also Reiss, 1996). The formation of the maxillopalatine is also variable and larval *Grandisonia* cf. *larvata*, *G. sechellensis* and *Praslinia cooperi* already have the maxilla and palatine fused. The overall morphology of the larval maxillopalatine in these taxa, however, is far more similar to the respective separate elements in the larvae of the other taxa investigated and the posterior growth of the maxillary part of the palatine occurs only at metamorphosis as in rhinatrematid, ichthyophiid, uraeotyphlid and

Sylvacaecilia grandisonae larvae. Caeciliid larvae further retain separate nasals and premaxillae, the fusion of which upon metamorphosis forms the nasopremaxilla of the caeciliid adult. Also present in larval caeciliids is a free septomaxilla that is otherwise only found in rhinatrematid, ichthyophiid, uraeotyphlid and scolecomorphid caecilians (Taylor, 1969; Wilkinson & Nussbaum, 2006). Previously, limited developmental evidence indicated a transitory and irregular occurrence of the septomaxilla during develoment in caeciliids and a subsequent incorporation into the nasopremaxilla (Marcus *et al.*, 1935; Wake & Hanken, 1982).

Larval caecilians also share a similar general morphology of the hyobranchial skeleton as compared to adults with some variation in the number of basibranchial elements between different taxa. Upon metamorphosis, the ceratohyal and ceratobranchial components become remodelled and fuse with the ceratohyal and ceratobranchial I. At the same time, ceratobranchial IV greatly reduces its size and fuses with ceratobranchial III. Larval *Grandisonia* show an early fusion of the ceratobranchial III and IV as compared to other caecilian larvae. Rhinatrematids, however, deviate from this common metamorphic pattern and exhibit a complex and profound remodelling of virtually the whole hyobranchial skeleton, in which bony elements are replaced by cartilage (Wake, 1989). Rhinatrematids further show a greater reduction in the number of arches in the adult hyobranchial skeleton than other caecilians (Nussbaum, 1977).

Larval caecilian musculature and its metamorphosis has so far been very poorly studied. Edgeworth (1935) commented on the musculature of larval *Ichthyophis*, Haas (2001) described the morphology of the trigeminus innervated adductor musculature in larval *I. kohtaoensis* and Kleinteich & Haas (2007) presented a detailed study of the entire cranial musculature of the same species. As with cranial and hyobranchial morphology, muscular morphology is also remarkably similar among larval caecilians. Several differences nonetheless exist between the investigated taxa. Rhinatrematids show the most elaborate development of the primary jaw adductor musculature in larvae, followed by ichthyophiids. Unique to rhinatrematid lavae is that the *m. interhyoideus posterior*

is not attached to the retroarticular process and shifts its insertion onto the retroarticular process only during metamorphosis. Rhinatrematids are further characterized by the pressence of a small, dorsal portion of the *m. levator hyoideus*, which resembles the levator muscles of the more posterior branchial arches and is otherwise present only in larval *Praslinia cooperi*. Upon metamorphosis, most of the musculature undergoes repatterning: the more lateral parts of the primary jaw adductor muscles are resorbed to make space for the anterior growths of the squamosal and the *m. adductor mandibulae externus*, where differentiated, disappears completely, certain other muscles such as the *m. levator hyoideus* and the *m. hyomandibularis* disappear and are probably partly incorporated into the *m. depressor mandibulae* and *m. pterygoideus*, respectively (Kleinteich & Haas, 2007).

Of special interest is the phylogenetic distribution of larvae. The presence of larvae in rhinatrematids, ichthyophiids and uraeotyphlids and outgroup comparison with frogs and salamanders (Duellman and Trueb, 1986) indicates oviparity with a free-living larva to be plesiomorphic in caecilians and their presence in the basal branching is taxa therefore not unexpected. The phylogenetic distribution of free-living larvae in caeciliids is somewhat more puzzling. *Praslinia cooperi*, *Grandisonia* cf. *larvata* and *G. sechellensis* are part of a monophyletic group of Seychellean caeciliids (Hedges *et al.*, 1993; Wilkinson *et al.*, 2003) that also contains species that develop directly (see Nussbaum, 1984)(Fig. 1; see also Chapter 4). Using character optimization on current phylogenies (Wilkinson et al., 2003, Frost et al., 2006; Roelants et al.; 2007) suggests the re-evolution of a free-living larva in these taxa, rather than a plesiomorphic retention. The discussion of the distribution of larvae in caeciliids is further complicated by the unknown phylogenetic position of *Sylvacaecilia grandisonae*, which could be expected to have a profound influence. A reversal from direct development to a free-living larva has recently been proposed for some plethodontid salamanders (Chippindale et al. 2004; Mueller et al. 2004). As pointed out by Müller *et al.* (in prep; see Chapter 4) caecilians are different from other amphibians in that large eggs and brood care are characteristic of all

oviparous species and seem to have evolved early during caecilian evolution. This suggests that the evolution of direct development in caecilians might have been more plastic than the ancestral state reconstructions suggest, with possibly a repeated independent evolution in various groups within higher caecilians rather than a re-revolution of a free-living larva. Evidence for this comes also from the morphology of larval *P. cooperi, S. grandisonae* and *Grandisonia*. Larval *P. cooperi* and *S. grandisonae* show numerous similarities with larval rhinatrematids, ichthyophiids and uraeotyphlids. Both have well developed *mm. hyomandibularis et m. levator hyoideus* and a robust hyobranchial skeleton. Especially *P. cooperi* is characterized by a large *m. levator hyoideus* that is furthermore divided into two portions as in larval rhinatrematids and also a similarly shaped squamosal as in rhinatrematids and ichthyophiids. It seems unlikely that these characters, which are exclusively larval in caecilians (adult typhlonectids, however, seem to have a *m. hyomandibularis*, Wilkinson & Nussbaum, 1997) have been re-evolved from a direct developing ancestor. The ontogeny of viviparous and direct developing species (Wake and Hanken, 1982; Müller *et al.*, 2005) provides further no indication for retention of some of these characters during early ontogeny that would facilitate a re-evolution of a free-living larva through heterochronic shifts. Larval *Grandisonia*, however, are more different in their morphology from other caecilian larvae in that they lack a *m. levator hyoidei*, have only a weakly developed *m. hyomandibularis* and fused ceratobranchials III and IV. In their skeletal characters, larval *Grandisonia* are remarkably similar to late embryos of the direct developing *Hypogeophis rostratus* (Müller, 2006; Chapter 2). Embryonic *H. rostratus* also have a very weakly developed *m. hyomandibularis* (HM, pers. obs.). Heterochronic shifts could possibly produce a free-living larva-like form derived from a *H. rostratus*-like, direct developing form. However, the alternative hypothesis, that *H. rostratus* only recently evolved direct development from a *Grandisonia*-like ancestor that has lost some of its larval characters, is equally parsimonious and seems also more plausible (see also Nussbaum, 1984, for data on caecilian ecology in the Seychelles).

Are caecilians primarily or secondarily stegokrotaphic?

Before turning to the discussion of stegokrotaphy *vs.* zygokrotaphy in caecilians, it seems necessary to take a look at the development of the temporal region in Palaeozoic, presumably plesiomorphically stegokrotaphic froms. Unfortunately very little data are available concerning the development and metamorphosis of the skull in Palaeozoic amphibians. However, Schoch (1992) described the development of the skull in *Apateon caducus* and *A. pedestris* based on a large and well resolved series of different ontogenetic stages. *Apateon* is a small branchiosaurid temnospondyl amphibian that was aquatic and neotenic, although rare occurrences of metamorphosis have been reported (Werneburg 1991, Schoch & Fröbisch 2006). The skull of *Apateon* is stegokrotaphic and the cheek region is formed by the squamosal posteriorly, the supratemporal and postfrontal dorsally, the postorbital and jugal anteriorly and the quadratojugal ventrally. In early developmental stages, only the squamosal, supratemporal and quadratojugal are present, whereas the remaining bones form slightly later (Fig. 20). The squamosal is relatively slender, with a pronounced posterior notch that gives it a distinct hook-shape in the earlier stages, not entirely dissimilar to that of larval rhinatrematids or ichthyophiids. During further development, the squamosal shows only some slight and gradual anterior expansion and the anterior cheek region is closed by the developing jugal and postorbital. At about the same time, dermal sculpturing begins to form on these elements and most of the other dermal skull bones and in particular the dorsal skull roofing bones, which is seen as an indication that the jaw adductor musculature did not extend onto the dorsal side of the skull as in rhinatrematid caecilians or living salamanders. The available, more limited data about the development of other Palaeozoic amphibians (e.g. Schoch, 2002a, 2002b; Witzmann & Pfretzschner, 2003; Witzmann, 2005) indicates a positive allometric growth of the squamosal during ontogeny similar to *Apateon*, but shows no evidence of the pronounced remodelling of the squamosal seen in caecilians upon metamorphosis.

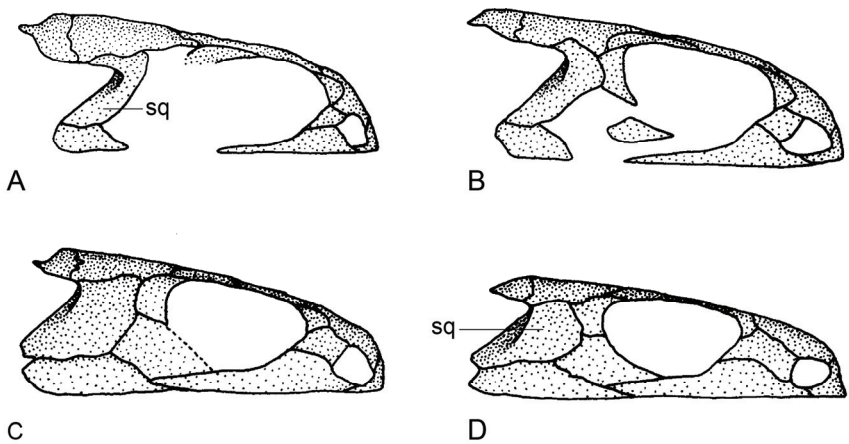

Fig. 20. Stages in the development of the skull in the branchiosaurid temnospondyl *Apateon pedestris* (redrawn from Schoch, 1992). Skull length in A is approximately 6 mm, 10 mm in B, 16 mm in C and 20 mm in D.

Carroll & Currie (1975) proposed an ancestry of caecilians among the lepospondyl microsaurs; more specifically they proposed a close relationship with the microsaur *Goniorhynchus*. Implicit in this hypothesis is that stegokrotaphy in Recent caecilians must be a plesiomorphic retention and zygokrotaphy secondarily evolved within caecilians. Nussbaum (1983) argued for stegokrotaphy to be secondarily derived within caecilians and presented four supporting lines of evidence. First, no bones of the temporal series are present between the parietal and squamosal in Recent caecilians, which is most parsimoniously explained by their loss due to temporal fenestration than in situ fusion of bones. Secondly, the arrangement of postorbital bones in stegokrotaphic caecilians is notably different from that of primarily stegokrotaphic Paleozoic amphibians. Thirdly, the skulls of the basal-most caecilians, rhinatrematids and ichthyophiids, are either zygokrotaphic or weakly stegokrotaphic, whereas marked stegokrotaphy is only found in most, but not all, species of the highly derived caeciliid caecilians. Fourthly, the relatively weak development of the secondary jaw closure mechanism in rhinatrematids and its increasing elaboration in higher caecilians is consistent with the idea that it evolved as a compensation

for the decrease in size of the primary jaw adductor musculature due to its increasing confinement to a newly formed adductor chamber.

Recently, Jenkins *et al.* (in press) presented a detailed description of the morphology of *Eocaecilia micropodia*. In this putative stem-line caecilian, the cheek region is completely covered by the jugal, quadratojugal, postfrontal, squamosal and a possible tabular and therefore very likely primarily stegokrotaphic. Jenkins et al. (in press) reviewed the arguments of Nussbaum (1983) against primary stegokrotaphy in living caecilians in the light of their finds in *E. micropodia*. Regarding the absence of temporal bones in living caecilians, Jenkins *et al.* (in press) noted that, citing Wake & Hanken (1982) and Wake (2003), living caecilians show extensive fusion of cranial bones. While the degree of fusion seen in the caecilian skull is certainly remarkable, it must be noted that neither Wake & Hanken (1982) nor Müller *et al.* (2005) or Müller (2006; see also Chapter 2) found any ontogentic evidence for bone fusions in the temporal area, but instead demonstrated that the squamosal is the only bone that forms in the temporal region, which originates from a single centre of ossification and grows rostrally during ontogeny to cover most of the cheek. Furthermore, Wake & Hanken (1982) regarded the absence of temporal elements other than the squamosal during ontogeny as explicit evidence for the secondary nature of stegokrotaphy in caecilians.

As for the second argument of Nussbaum (1983), Jenkins *et al*. (in press) stated that the architecture of the cranial vault of *E. micropodia* includes elements from the postorbital and temporal series in positions comparable to those of Palaeozoic amphibians. This however, provides evidence only for the primary stegokrotaphy of *E. micropodia* but not for Recent caecilians, which, as argued above, have lost most of the elements found in the cheek region of *E. micropodia*.

Regarding Nussbaum's (1983) third line of evidence, Jenkins *et al.* (in press) argue that rhinatrematid caecilians exhibit a number of derived characters in their cranial osteology, i.e. the loss of pre- and postfrontals, the position of the orbit entirely within the maxillopalatine and the specialization of the articulation of the squamosal with the lateral margin of the os basal, whereas ichthyophiids

(and uraeotyphlids) show "more primitive character states in these features". Carroll (2000) also considered Ichthyophiidae and Uraeotyphlidae to be "the most primitive living caecilians", based on the observed trend towards loss and fusion of skull bones among caecilians. However, although rhinatrematids show apparently apomorphic conditions in the expression of the abovementioned characters, in no way can this be taken to indicate that most other, if not all, characters of rhinatrematids are therefore also derived. Numerous phylogenetic studies have repeatedly and consistently resolved rhinatrematids as the sistergroup to all other living caecilians (e.g. Nussbaum, 1977; Wilkinson, 1992b; Wilkinson, 1996; Hedges *et al.*, 1993; San Mauro *et al.*, 2004, 2005; Roelants *et al.*, 2007). Rhinatrematids exhibit furthermore a large number of plesiomorphic characters, apart from the presumably plesiomorphic configuration of the skull and associated musculature. These include the possession of haemal arches in larvae (Carroll et al., 1999), heart morphology (Wilkinson, 1996) and the morphology of the lateral line system (Wilkinson, 1992b), among others. While these plesiomorphic characters alone, as argued above, do not justify the assumption that rhinatrematids exhibit a plesiomorphic condition in all characters, the large number of clearly plesiomorphic characters together with their basal-most branching position nonetheless supports the assumption that rhinatrematids are probably more similar to the last common ancestor of living caecilians than other representatives of the group. *Epicrionops* and *Rhinatrema* show a deep divergence and long independent evolutionary history (Gower *et al.*, 2002; Roelants *et al.*, 2007), as indicated by profound differences such as in hyobranchial (Nussbaum, 1977) or cloacal (Taylor, 1968) morphology. Both genera are nonetheless very similar in their skull morphology and cranial musculature, which is seen here as a further indication of common descent from a form close to the last common ancestor of living caecilians.

Lastly, Jenkins *et al.* (in press) suggested that the weak development of the secondary jaw closure mechanism in rhinatrematids might be the result of a secondary reduction and a concomitant compensatory enlargement of the primary jaw adductors, which implies a secondarily evolved zygokrotaphic skull. This

argument, however, implies a causal link between the length of the retroacticular process and the sizes of the primary jaw adductor musculature and the *m. interhyoideus posterior*. It is evident that the secondary jaw closure mechanism is less developed in rhinatrematids than in other caecilians, which show a clear trend towards the enlargement of the retroarticular process and the *m. interhyoideus posterior* and a reduction of the primary jaw adductor musculature. The function of the retroarticular process as an attachment and lever arm for the *m. interhyoideus posterior*, which functions as a secondary jaw closure muscle in Recent caecilians, is also indisputable (Nussbaum, 1977; 1983; Bemis *et al.*, 1983) and both are negatively correlated with the size of the primary jaw adductor musculature. However, the elongation of the retroarticular process might have been initially unrelated to the secondary jaw closure mechanism seen in living caecilians. A retroarticular process is found in a number of amphibians and reptiles, such as the amphiumid salamander *Amphiuma* (albeit short; Erdman & Cundall, 1984), crocodiles (Iordansky, 1973) and varius other reptiles such as the nothosaurid *Pachypleurosaurus* (Carroll & Gaskill, 1985), all of which are comparatively long-snouted and aquatic. An elongated retroarticular process, which serves as the attachment site of the *m. depressor mandibulae*, might greatly facilitate jaw opening in an aquatic environment where the higher density of the surrounding medium requires a stronger force for jaw opening than on land. A similar reason might be the case of the elongation of the retroarticular process in caecilian evolution, where a stonger force for jaw opening is not only required during their aquatic larval phase but also on land, where a stronger force might also facilitate jaw opening in a subterranean habitat. In rhinatrematids, the retroarticular process of the lower jaw is comparatively short and the *m. interhyoideus posterior* is also comparatively smaller than in other caecilians. Also, in contrast to all other caecilians, the *m. interhyoideus posterior* does not attach to the retroarticular process in larval rhinatrematids and therefore does not function as a jaw closing muscle. Only during metamorphosis does the *m. interhyoideus posterior* shift its insertion from the ceratohyal onto the retroarticular process and subsequently functions as a jaw closing muscle.

Assuming a secondary reduction in the secondary jaw closure mechanism as proposed by Jenkins *et al.* (in press) does not explain the shift in insertion of the *m. interhyoideus posterior* during metamorphosis, which would be supposedly also secondary. It seems more plausible to accept the alternative explanation that larval rhinatrematids retain the ancestral state of the configuration of the *m. interhyoideus posterior*, which is only subsequently incorporated into the secondary jaw closure mechanism during metamorphosis. This scenario is also consistent with the hypothesis favoured here that the relatively weak development of the secondary jaw closure mechanism in rhinatrematids, as compared to other caecilians, represents a plesiomorphic condition rather than being secondarily derived. Implicit in this assumption is that the large primary jaw adductor musculature of rhinatrematids that exends onto the dorsal side of the skull is also plesiomorphic within caecilians.

Carroll & Holmes (1980), Carroll (2000) and Jenkins *et al.* (in press) argued that temporal fenestration evolved probably independently in frogs, salamanders and caecilians because different adductor muscles exit through the temporal opening onto the dorsal side of the skull in all three groups. According to Carroll & Holmes (1980) and Carroll (2000), the muscle that extends onto the dorsal side of the skull is the longus head of the *m. adductor mandibulae posterior* in frogs, the superficial head of the *m. adductor mandibulae internus* in salamanders, and the *m. adductor mandibulae externus* in rhinatrematid caecilians. Haas (2001), in a thorough study of jaw adductor muscles in particularly frogs but also some salamanders and larval *Ichthyophis* (see also Kleinteich & Haas 2007), concluded that the previous system of homologizing jaw adductor muscles based on their position in relation to the trigeminal nerve branches is flawed and proposed new homologies and presented a consistent nomenclature of amphibian jaw adductor muscles. According to his sheme, which is followed here, the same muscle, the *m. adductor mandibulae longus*, extends onto the dorsal side of the skull in all three groups of living amphibians. While this does not exclude the possibility of an independent evolution of skull fenestration in frogs, salamanders and caecilians, it nonetheless shows remarkable

similarity in the arrangement in the jaw adductor musculature with regard to the temporal opening in all three groups. Of further interest is the position of the *m. adductor mandibulae longus* in relation to the *m. depressor mandibulae* in larval *Ichthyophis*, where the superficial head of the *m. adductor mandibulae longus* overlaps the *m. depressor mandibulae* dorsally, as in larval rhinatrematids.

It is possible to provide arguments for both stegokrotaphy being ancestral or derived in caecilians and a more complete fossil record would certainly help to illuminate the evolution of the caecilian skull. In this study, we have focused on the morphology of the skull and associated musculature of Recent larval caecilians and their metamorphic transformation. Larval caecilians resemble larval and most adult salamanders as well as most adult frogs in that the skull is gymnokrotaphic (Trueb, 1993), with the squamosal being the only dermal skull roofing bone in the temporal region (some frogs and salamanders, however, do possess a small quadratojugal [Lebedkina, 1979; Trueb, 1993]). The larvae of rhinatrematids, ichthyophiids and, to a lesser extent, *P. cooperi* show an even stronger resemblance to other living non-caecilian amphibians in that the squamosal connects the quadrate to the parietal and/or otic capsule to provide structural support to the suspensorium (Carroll & Holmes, 1980; Trueb, 1993; Rose, 2003). Larval rhinatrematids and ichthyophiids further have a well developed primary jaw adductor musculature in which the superficial layer of the *m. adductor mandibulae longus* extends onto the dorsal side (or dorsolateral side in ichthyophiids) of the skull and overlaps the *m. depressor mandibulae*. All caecilian larvae have a pronounced posterior elongation of the maxilla during metamorphosis, which is also found in salamanders and frogs but not known from any Palaeozoic amphibian group, and which has been suggested to be a lissamphibian synapomorphy (Reiss, 1996, 2002). Rhinatrematids, and to a lesser degree also ichthyophiids and uraeotyphlids, show also a pronounced remodelling of the palate during metamorphosis (Reiss, 1996), which is reminiscent of palatal metamorphosis in salamanders (Rose, 2003), although the pattern of remodelling is not completely congruent between caecilians and salamanders because the bones of the palate are differently affected. The large number of shared

similarities of Recent amphibians and the different formation of the bony cheek in caecilians and palaeozoic amphibians, together with the arguments presented before, make a secondary evolution of stegokrotaphy in caecilians more plausible than the alternative hypothesis.

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Appendix 1

Taxon	Number	Stage	Total length (in mm)	Preparation	Locality
Epicrionops lativittatus	UMMZ 185861	larva	109	dissection, c&s	Ecuador: Cotopaxi: San Francisco de las Pampas
	UMMZ 185865	larva	157	dissection, c&s	"
	UMMZ 185826	larva	-	c&s	"
	UMMZ 185871	larva	175	dissection, c&s	"
	UMMZ 185856	larva	173	dissection, c&s	"
	MNHG 2554.44	subadult	209	dissection, c&s	"
	UMMZ 188835	adult	-	dissection, c&s	"
Rhinatrema bivittatum	UMMZ uncatalogued	larva	-	dissection	French Guiana
	BMNH uncatalogued	adult	-	serial sections	"
Ichthyophis bannanicus	UMMZ 190503	larva	110	dissection, c&s	China: Yunnan: Mengla
	UMMZ 190250	larva	117	c&s	"
	UMMZ 190252	larva	156	c&s	"
	UMMZ 190454	larva	182	c&s	"
	UMMZ 190246	larva	186	dissection, c&s	"
	UMMZ 205185	adult	289	dissection, c&s	"
Ichthyophis cf. *kohtaoensis*	BMNH uncatalogued	larva	67	dissection, c&s	Thailand: Na Sabaeng,
		larva	112	dissection, c&s	"
		metamorph	146	dissection, c&s	"

		metamorph	158	dissection, c&s	"
		juvenile	187	dissection, c&s	"
		adult	319	dissection, c&s	"
Uraeotyphlus cf. *naranyani*	MW 00355	hatchling larva	57	dissection, c&s	India
	MW 00739	adult	234	dissection, c&s	"
Uraeotyphlus oxyurus	BMNH 82.12.12.11	larva	-	dissection, c&s	India
Grandisonia cf. *larvata*	BMNH 80.10.22.14	larva	49	dissection, c&s	Seychelles
Grandisonia sechellensis	UMMZ 221145	hatchling larva	32	dissection, c&s	Seychelles
	UMMZ 200567	larva	61	dissection, c&s	"
	UMMZ 195769	adult	179	dissection, c&s	"
Praslinia cooperi	UMMZ 182990	larva	117	dissection	Seychelles: Mahé
	UMMZ 179957	larva	119	dissection, c&s	"
	BMNH 1910.3.18.86	adult		c&s	"
	UMMZ 175392	adult	218	dissection	"
Sylvacaecilia grandisonae	BMNH 1972.990	larva	77	dissection	Ethiopia: Illubabor: Abiu
	BMNH 1972.986	larva	99	dissection, c&s	"
	BMNH 1976.1131	juvenile	116	c&s	"
	BMNH 1972.981	subadult	139	dissection	"
	BMNH 1972.980	adult	249	dissection, c&s (disarticulated)	"

CHAPTER 4

Heterochrony, ontogenetic repatterning, and the evolution of direct development in caecilian amphibians

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ABSTRACT Direct development is one of the most striking developmental adaptations in amphibians, involving heterochrony and ontogenetic repatterning. Despite it being of universal importance in amphibians – direct development is present in all three living major groups (frogs, salamanders and caecilians) – very little detailed information is available on direct development in caecilians. We here describe the postembryonic development of the skull in *Boulengerula taitanus*, a direct developing caecilian with an extended period of post-hatching parental care. Postembryonic skull development is compared with that of *Gegeneophis ramaswamii*, a direct developing species without post-hatching parental care, and *Ichthyophis* cf. *kohtaoensis*, a species with a free-living aquatic larva. Compared with *G. ramaswamii*, hatchling *B. taitanus* have a far less developed skull and are unlikely to be able to burrow. Skull development, especially the closure of the cheek region continues during the early postembryonic phase. The general trajectory of skull development in *B. taitanus* is nonetheless similar to that of *G. ramaswamii*, indicating a heterochronic shift in hatching time in the former. Skull development in both species is further characterized by the absence of larval-specific traits seen in larvae of *I.* cf. *kohtaoensis*, which shows that direct development in caecilians is also characterised by ontogenetic repatterning.

INTRODUCTION

One of the classic characteristics of amphibians is the possession of a complex life-cycle that is characterized by a free-living larval stage, which undergoes a complex transformation process (metamorphosis) into the adult (Duellman and Trueb 1986). However, this belies the great diversity of amphibian life-histories and associated ontogenies that have evolved within the group. The biphasic life-cycle of amphibians, with a primarily aquatic larva followed by a primarily terrestrial adult, offers many opportunities for natural selection to act upon. Depending on environmental conditions, an evolutionary advantage should be gained by extending or shortening the aquatic larval phase as opposed to the terrestrial adult, or by maintaining the equilibrium between the two. At the two

extremes of this trajectory lie paedomorphosis – sexual maturity is attained by the larva, which fails to undergo a metamorphosis and remains permanently aquatic (e.g. Duellman and Trueb 1986; Denoël et al. 2005) – and direct development – a free-living larva is absent from the ontogeny and an adult-like juvenile hatches out of a terrestrially deposited egg (e.g. Wake and Hanken 1996). Between these two extremes, many different reproductive strategies have evolved in all three orders of living amphibians (Anura, Caudata and Gymnophiona) that differ widely in, for example, egg deposition sites, larval habitat, duration of the larval phase and parental care (see e.g., Nussbaum 1985; Duellman and Trueb 1986, Prado and Haddad 2005).

Direct development, heterochrony and ontogenetic repatterning

Among the plethora of developmental adaptations and modifications, direct development is one of the most fascinating evolutionary innovations. In virtually all major groups of animals, several lineages have developed ways to circumvent the free-living larval stage and transform more or less directly into an adult-like organism (e.g. Raff and Wray 1989; Wake and Hanken 1996; Collin 2004). Direct development in amphibians, as the name suggests, is characterized by the absence of a free-living, usually aquatic larva. Most adult features that do not form until metamorphosis (or later) in transforming species (Hanken et al. 1992; Wake and Hanken 1996) develop already during the embryonic period, resulting in, for example, the hatching of a small, but more or less fully formed frog rather than a tadpole.

Direct development appears to be an evolutionary successful strategy. It is a widespread phenomenon and known to occur in all three orders of modern amphibians. Within frogs, direct development has evolved independently in several groups (e.g. Thibaudeau and Altig, 1999, Bossuyt and Milinkovitch 2000; Müller et al. in press) and several authors have suggested that direct development was the key evolutionary innovation that enabled the radiation of the mega-diverse leptodactylid genus *Eleutherodactylus*, the rhacophorid *Philautus* and other groups (e.g., Hanken et al. 1992; Meegaskumbura et al. 2002). In

salamanders, direct development occurs only within the lungless plethodontid salamanders, but might have evolved several times in this group (Wake and Hanken 1996, Chippendale et al. 2004; Mueller et al. 2004). Direct developing plethodontids, however, make up the majority of all living salamanders and the evolution of direct development has been implicated in both their evolutionary success in terms of species numbers, and the dispersal of bolitoglossine plethodontids into tropical Central and South America (Wake & Hanken, 1996).

The evolution of direct development is not well understood. It is generally assumed that it evolves through an increasing terrestrialisation of development as a way of bypassing the aquatic environment. Although the ancestral biphasic life-history has a tremendous potential for adaptive diversification (e.g. Wassersug 1975; Hanken et al. 1997; Hoff et al. 1999), living in aquatic habitats also carries a significant risk due to possible desiccation and predation. Direct development can be viewed as adaptive in terms of being able to ensure reproductive success by controlling the timing of reproduction to avoid desiccation, as well as through prolonging embryonic development (leading to developmentally more advanced offspring) and/or decreasing the time spent in the aquatic environment (e.g. Duellman and Trueb, 1986, Haddad and Prado, 2005).

Developmentally, two mechanisms are primarily involved in the evolution of direct development: heterochrony and repatterning. Heterochrony leads to a shift in the on- or offset of events during development as compared to the ancestral ontogeny (e.g., Alberch et al. 1979; Raff and Wray 1989; Reilly et al. 1997). In *Eleutherodactylus coqui*, the majority of bones forming the skull are already present at hatching, whereas they do not form until metamorphosis in biphasic anurans (Hanken et al. 1992). However, in *E. coqui*, there is not only a heterochronic shift of the larval into the embryonic period, such that the tadpole would develop inside the egg and undergo a metamorphosis before hatching. Instead, the embryonic development is characterized by large scale ontogenetic repatterning: the development is highly modified (Townsend and Stewart 1985) and results in a loss of most larval-specific features and a precocious formation of the adult morphology (Ellinson, 1990; Hanken, 2003). Many regions of the skull

assume a postmetamorphic, adult-like morphology from their inception, while other structures, such as the hyobranchial skeleton, initially assume a mid-metamorphic morphology (Hanken et al. 1992). This is also reflected in the development of the jaw adductor musculature, which initially assumes a mid-metamorphic configuration and larval-type myofibres are entirely absent (Hanken et al. 1997). Ontogenetic repatterning also seems to characterize the development of direct-developing bolitoglossine salamanders, where the hyobranchial skeleton forms in an adult-like configuration during embryogenesis (Alberch 1987).

Direct development in caecilian amphibians

All major reproductive modes – biphasic, direct development and viviparity – are found in caecilian amphibians. Free-living, largely aquatic larvae occur in rhinatrematid, ichthyophiid, uraeotyphlid and some caeciliid caecilians (Sarasin and Sarasin 1887-1890; Parker 1958; Largen et al. 1972; Wilkinson and Nussbaum 1996), indicating that this is the ancestral condition for the group (Fig. 1). Viviparity occurs in scolecomorphids, typhlonectids and some caeciliids (e.g. Peters 1875; Barbour and Loveridge 1928; Parker 1936; Taylor 1968). Direct development seems to be restricted to caeciliids, but very little information is available beyond the fact that a larval stage is seemingly absent in several species. Direct development is known, or is suspected to occur, in *Boulengerula taitanus* (Nussbaum and Hinkel 1994; Malonza and Measey 2005), *Caecilia orientalis* (Funk et al. 2004), *Gegeneophis ramaswamii* (Müller et al. 2005), some species of *Grandisonia* (Wake 1977), *Hypogeophis rostratus* (Brauer 1897), *Idiocranium russeli* (Sanderson 1937) and *Siphonops annulatus* (Goeldi 1899; Jared et al. 1999).

Little information is available regarding the mechanisms or consequences of direct development in caecilians. An aquatic life-history stage is apparently absent and juveniles of the abovementioned species are fully terrestrial upon hatching. This implies that most, if not all, of the characters thought to be associated with a burrowing life-style in caecilians, such as a well-ossified skull and the chemo- and mechanosensory tentacle (Himstedt 1996 and references

therein) are fully functional upon hatching. This indeed seems to be the case, based on the limited evidence available. Brauer (1899) described the precocious development of the tentacle in embryonic *Hypogeophis rostratus*, a character known to develop only during metamorphosis in larvae of ichthyophiid caecilians (Sarasin and Sarasin 1887-1890; Dünker et al. 2001). Müller et al. (2005) and Müller (2006; Chapter 2) described the development of the skull in the direct developing *Gegeneophis ramaswamii* and *Hypogeophis rostratus*, respectively, and several characters in both species show signs of precocious development as compared to free-living larvae of biphasic species. For example the maxilla and palatine fuse well before hatching to form the maxillopalatine, a compound bone characteristic of adult caecilians that forms during metamorphosis in biphasic species. Both species have a very similar ossification sequence of the skull, which is different from that of the viviparous *Dermophis mexicanus* (Wake and Hanken 1982; Müller et al. 2005; Müller 2006). Hatchling *G. ramaswamii* furthermore have a well-developed skull that resembles the adult condition in that species. The skull morphology of hatchling *H. rostratus* is unknown.

That direct development in caecilians is more diverse than previously thought is demonstrated by recent discoveries in *Boulengerula taitanus*. Recently, Malonza and Measey (2005) reported *B. taitanus* to have altricial young that are seemingly unable to burrow in soil. Kupfer et al. (2006; see Chapter 5) established that hatchling *B. taitanus* are very small (around 28 mm) compared to those of other direct developing caecilians (Brauer 1899; Müller et al. 2005) and receive extended parental care from the guarding female, in the form of feeding of their offspring with their own modified skin. Kupfer et al. (2006; Chapter 5) further noted that juveniles will stay with their mother until a length of about 86 mm, by which time they resemble miniature, albeit less pigmented adults.

To gain a better understanding of direct development in caecilians, we investigated its impact on the postembryonic morphology in *G. ramaswamii* and *B. taitanus*, focussing on the development of the skull as a particularly rich source of characters known to be influenced by heterochronic shifts associated with direct development in other amphibians (Hanken et al. 1992). Postembryonic

skull development in the two direct developing species is compared with *Ichthyophis* cf. *kohtaoensis*, which has a free-living aquatic larval stage.

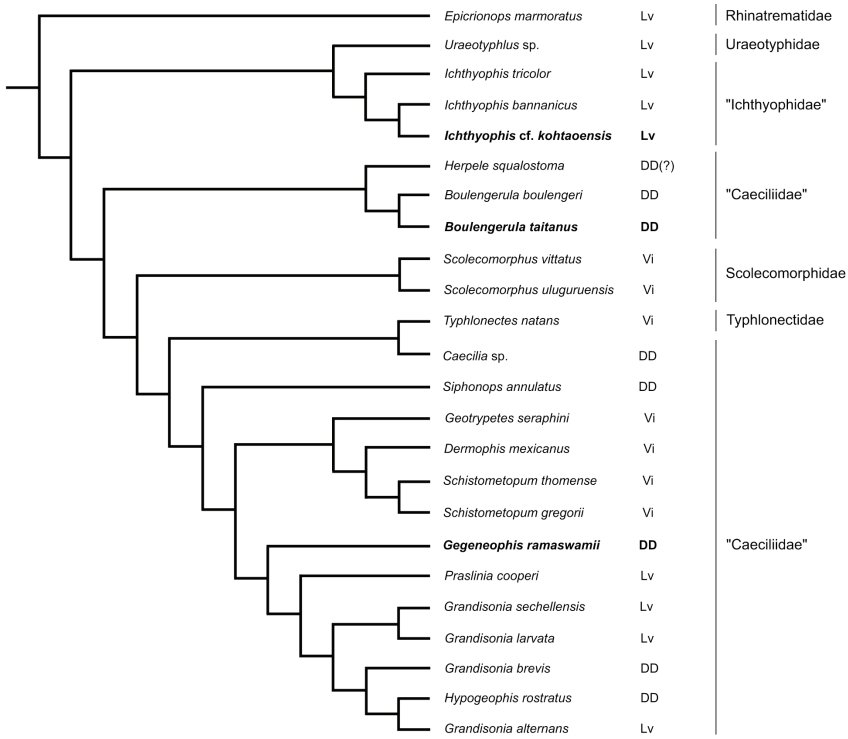

Fig. 1. Phylogenetic relationships of caecilians, from Wilkinson et al. (2003), modified according to Gower et al. (2002) regarding *Ichthyophis* spp. relationships. Taxa in investigated here are in bold face; note that taxon "16 *Ichthyophis* sp." of Gower et al. (2002) corresponds to *I.* cf. *kohtaoensis* in this study. Lv – biphasic with a free-living larva, DD – direct development, Vi – viviparity.

MATERIAL AND METHODS

Available for this study were ontogenetic series ranging from embryos to large adults of the indirect developing *Ichthyophis* cf. *kohtaoensis*, and the direct developing *Boulengerula taitanus* and *Gegeneophis ramaswamii*. Specimens of *G. ramaswamii* were collected from Kerala, India (for further details see Müller et al. 2005), *B. taitanus* from the vicinities of Wundanyi and Maghimbinyi, Taita Hills, Kenya, and *I.* cf. *kohtaoensis* from the vicinity of Na Sabaeng village, Khemmarat District, Ubon Rathchathani Province, Thailand (for further details

on locality and egg collection see Kupfer et al. 2004). Specimens were either fixed in the field or reared in the lab from field collected clutches and fixed in Bouin's fluid, 4% neutral buffered formalin or 70% ethanol and all subsequently stored in 70% ethanol. Embryos of *I.* cf. *kohtaoensis* and *B. taitanus* were removed form their egg capsules prior to fixation. Specimens are housed in the herpetological collections of the Natural History Museum, London (BMNH). For specimens details see Appendix.

Staging

Little information is available on the development of Gymnophiona. The only available staging tables are for *Typhlonectes compressicauda* (Sammouri et al. 1990), a highly derived viviparous species, and *Ichthyophis kohtaoensis* (Dünker et al. 2001), the latter of which was used for staging of *Ichthyophis* cf. *kohtaoensis* embryos and larvae used here. Both staging tables proved inadequate for the description of development in *B. taitanus* and *G. ramaswamii*, because several of the stage-defining characters (e.g., development of lateral line organs in the *I. kohtaoensis* staging table) are not expressed in embryos of these species. Embryos of *B. taitanus* and *G. ramaswamii* were therefore staged according to Brauer (1899). Brauer's description is more of an overview of the external development in *Hypogeophis rostratus*, a direct-developing Seychellean caeciliid, rather than a staging table in a modern sense, but his detailed figures and descriptions have subsequently been referred to as stages and can be used as such (see Müller 2006; Chapter 2).

Specimen preparation and investigation

Embryos of *B. taitanus* and *G. ramaswamii* have very little pigment and were usually surface stained with Borax carmine to enhance contrast for the examination of external characters, if necessary. Specimens used for the analysis of skeletal development were skinned and eviscerated (with the exception of embryos and very small specimens) and cleared and stained using standard procedures (Taylor & van Dyke 1985). All specimens were investigated under a

Nikon SMZ-U stereomicroscope equipped with a camera lucida and a Nikon Coolpix 995 for digital image capture.

Reconstruction of ancestral reproductive modes

The most recent comprehensive studies on caecilian intrarelationships (Wilkinson et al. 2003; Frost et al. 2006; Roelants et al. 2007) were used to establish most parsimonious optimizations of reproductive modes on the given phylogenies. Optimizations were calculated using the modular ancestral state reconstruction packages within the Mesquite system for phylogenetic computing (Maddison and Maddison, 2004). Reproductive mode was treated as a single character with three discrete character states (biphasic, direct developing, viviparous) that were treated as unordered or ordered in separate analyses. Information on reproductive modes was taken from Wake (1977) and Wilkinson and Nussbaum (1998), and references cited in the introduction to this paper. *Praslinia cooperi* was scored based on the assumed presence of larvae (Nussbaum and Gerlach 2004). Generally, knowledge about caecilian reproductive biology is still in its infancy and some of the assigned reproductive modes might prove incorrect.

RESULTS

External morphology of embryos

Embryos of *Boulengerula taitanus* and *Gegeneophis ramaswamii* share several features indicative of a direct mode of development (Fig. 2). In both species, the tentacle appears early during development and is clearly visible in older embryos. This is in contrast to *Ichthyophis* cf. *kohtaoensis*, where only a weakly developed tentacle anlage is present at hatching, which develops fully only during metamorphosis. Both direct developing species further show no lateral line organs expressed in their early development and also lack a well-developed gill slit and tail fin, all of which are characteristic for embryos and larvae of *I.* cf. *kohtaoensis*. At a comparable stage of development based on external features (e.g. gill differentiation), embryos of *B. taitanus* and *G. ramaswamii* are considerably smaller than those of *I.* cf. *kohtaoensis*, associated with larger egg,

hatchling and adult sizes in the latter (Taylor 1968; Kupfer and Müller 2002; Kupfer et al. 2004).

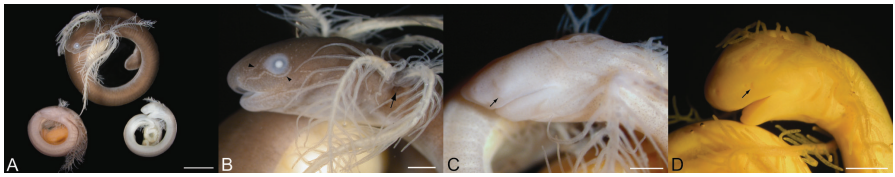

Fig. 2. A. Embryos of *Ichthyophis* cf. *kohtaoensis* (top), *Gegeneophis ramaswamii* (bottom left) and *Boulengerula taitanus* (bottom right) at a comparable stage of development with regard to the remaining yolk. The embryo of *G. ramaswamii* has been fixed inside the egg capsule and is more coiled than that of *B. taitanus*, which makes it appear smaller than it is. Scale bar equals 5 mm. B close-up of the head of the embryo of *I.* cf. *kohtaoensis* figured in A. Arrow heads point to the lateral lines and the arrow to the gill slit, note also the lip folds. C close-up of the head of the embryo of *G. ramaswamii* figured in A. Arrow points to the tentacle. D close-up of the head of an embryo of *B. taitanus*, slightly younger than the one figured in A. Arrow points to the developing tentacle. Scale bars in B, C and D equal 1 mm.

Postembryonic skull development in *Ichthyophis* cf. *kohtaoensis* and *Gegeneophis ramaswamii*

Postembryonic development in *Ichthyophis* cf. *kohtaoensis* includes a free-living, primarily aquatic larva that subsequently metamorphoses into a terrestrial, burrowing juvenile. Larval and adult skulls of different *Ichthyophis* species are very similar and have been described by several workers (e.g., Sarasin and Sarasin 1887-1890; Visser 1963; Wake 2003). Larval *Ichthyophis* cf. *kohtaoensis* are characterized by a short maxilla that is anterior to, and separate from, the palatine. The cheek region is not covered by bone and the squamosal is sickle-shaped, extending from the lateral side of the quadrate posteriorly onto the parietal, just in front of the otic capsule (Fig. 3). During metamorphosis, the maxilla starts to extend posteriorly, almost reaching the level of the posterior edge of the frontal in the adult. The maxilla further fuses with the palatine to form the maxillopalatine bone characteristic of all adult caecilians (Wake 2003), and its dental ridge extends posteriorly in parallel along the entire length of the dental ridge of the palatine part of the maxillopalatine. At about the same time, the dorsoposterior process of the squamosal that attaches to the parietal disappears, and the part of the squamosal lateral to the quadrate expands anteriorly to cover the cheek region. In the adults of most caecilians (Taylor 1969), the entire cheek

region is covered by the squamosal, which contacts the parietal dorsally and the maxillopalatine ventrally. The maxillary part of the maxillopalatine will also form a canal for the tentacle, which starts migrating at the beginning of metamorphosis and presumably becomes functional around this time. The lateral wall of this tentacular canal can either be closed or open, depending on the position of the tentacle and species (Taylor 1969). In adult *I.* cf. *kohtaoensis*, the tentacular canal is closed laterally.

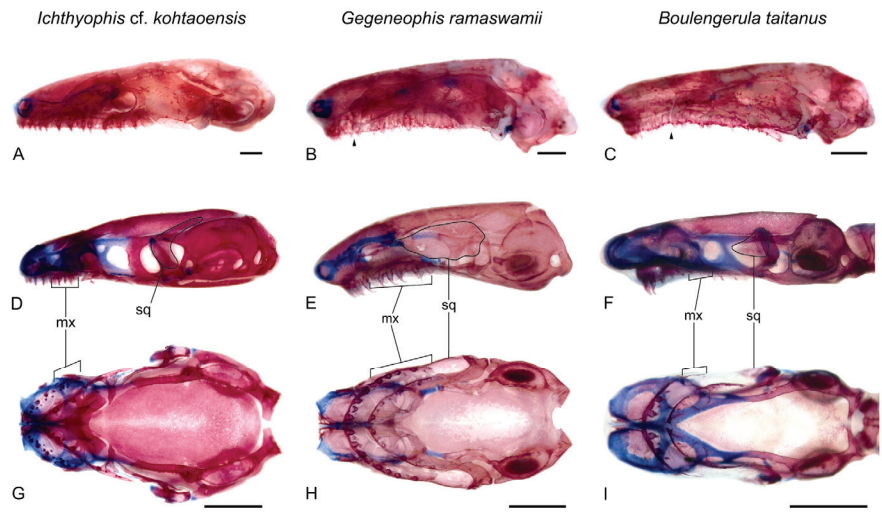

Fig. 3. Comparison of adult and hatchling skulls in *Ichthyophis* cf. *kohtaoensis*, *Gegeneophis ramaswamii* and *Boulengerula taitanus*. A B and C adult skulls. Arrow heads point to the posterior end of the premaxillary part of the nasopremaxillary. Lateral (D, E, F) and ventral view (G, H, I) of the skull of hatchling *I.* cf. *kohtaoensis*, *G. ramaswamii* and *B. taitanus*. The length of the dental lamina of the maxilla (mx) is indicated and outlines indicate the shape of the squamosal (sq) in lateral view. Scale bars equal 1 mm.

As in *Ichthyophis* cf. *kohtaoensis*, female *Gegeneophis ramaswamii* do not seem to provide care to their offspring for any extended period after hatching (DJG, MW pers. obs.; O. V. Oommen pers. comm.). Unlike for *Boulengerula taitanus* and *I.* cf. *kohtaoensis*, no *G. ramaswamii* specimen was available for which hatching was directly observed. One specimen, still encapsulated in the egg but with fully resorbed external gills, appears to be very close to hatching and has been described in detail by Müller et al. (2005). This specimen has an essentially adult-like morphology. The endocranium is well-ossified except for

parts of the nasal capsule, and all dermal elements are almost adult-like: nasal and premaxilla are fused and form the nasopremaxillary; maxilla and palatine are also fused, with the maxillary arcade extending backwards to the level of the posterior edge of the frontal bone. The cheek region is completely covered by the squamosal, which is only separated by relatively narrow gaps from the maxillopalatine, frontal and parietal (Fig. 3). The only difference to the adult skull is an open tentacular groove in the maxillopalatine, which closes during further development to form the tentacular canal, and a weakly ossified central area of the parasphenoid part of the os basal. Two additional *G. ramaswamii* specimens of similar and even slightly smaller size, which were found unaccompanied by adults or other juveniles (see Table 1), show more advanced development of the skull than the previous specimen. In both specimens the squamosal and maxillopalatine are in closer contact and the tentacular groove is almost closed, with just a narrow gap remaining between the dorsal and ventral parts of the maxillopalatine. The parasphenoid part of the os basal is fully ossified. Endocranial ossification is also more advanced, with cartilage restricted to the anterior copula of the nasal cartilage, the orbitonasal, cartilage and the orbital and trabecular cartilage, similar to the adult condition. Although hatching has not been observed in *G. ramaswamii* and the exact developmental stage of the skull at hatching is therefore unknown, it seems certain that the skull at hatching is more or less adult-like in its morphology apart from the tentacular groove. Some variation in hatching size is also likely to occur, as is indicated by the three smallest available specimens, and some variation in the degree of development of the skull might also exist. The tentacular groove is fully closed in a specimen of 85 mm total length but a specimen 100 mm total length still retains a narrow suture in the maxillopalatine. Another specimen of 101 mm, however, has the tentacular groove completely closed as in all other, larger specimens examined.

Embryonic and postembryonic skull development in *Boulengerula taitanus*

A *Boulengerula taitanus* embryo of Brauer stage 45 is externally similar to embryos of *Gegeneophis ramaswamii* of the same stage (Müller et al. 2005), but

is far less advanced in its skeletal development. The endocranium is well chondrified and appears slightly more robust than in *G. ramaswamii*, but unossified. Of the dermal ossifications, only premaxilla, vomer, palatine, parietal, dentary and angular are present, of which the premaxilla and dentary are the most prominent. The hyobranchial skeleton is only weakly chondrified but resembles that of Brauer stage 45 *G. ramaswamii* (Müller et al. 2005).

Two hatchling *B. taitanus* of 28 mm and 30 mm total length, respectively, were available for study. Both are at a similar, comparatively early stage of development. The entire endocranium anterior to, and including, the pila antotica is cartilaginous except for a very small, perichondral sphenethmoid ossification (Fig. 3). The footplate of the stapes has a thin perichondral ossification. All of the dermal bones are present, but poorly developed. The nasal and premaxilla are narrowly fused at the snout tip, via the alary process of the premaxilla, but widely separated laterally. The frontal and parietal are very thin and weakly ossified, and widely separated from their antimeres, leaving the brain exposed between them. Ventrally, the parasphenoid is very poorly developed, the anterior and posterior parts of the parasphenoid are still widely separated and only the posterior part of the parasphenoid is narrowly fused to the basal plate posteriorly, thus initiating the formation of the os basal (the compound bone formed from the posterior endocranium and parasphenoid). The maxilla is only a narrow sliver of bone and separate from the palatine. Maxillary teeth are absent. The squamosal is a small, triangular plate of bone lateral to the quadrate, and does not reach the pila antotica anteriorly. The premaxilla is comparatively large and is the only tooth bearing bone that has a well developed dental lamina with two fully developed teeth with ankylosed pedicels on each side. The palatine also bears two ankylosed teeth, but much smaller than the premaxillary teeth, while the vomer lacks ankylosed teeth. Some variation exists between the two hatchlings in the extent as to which the premaxilla covers part of the underside of the rostrum and in the development of the parts of the parasphenoid.

In a specimen of 35 mm total length, development of the dermal and endoskeletal ossification has generally progressed. The anterior and posterior

parts of the parasphenoid have fused but still leave a large central area unossified. Maxilla and palatine are still separate and the nasal and premaxilla are also still separated laterally. By 37 mm total length, the maxilla is partly fused to the palatine at its anterior end and a well-developed maxillary tooth is present, but not yet ankylosed. Nasal and premaxilla are fused laterally and the parasphenoid is more extensively fused to the endocranium ventral to the otic capsules, and shows a reticulate ossification pattern in its centre, with numerous interconnected bone trabeculae. The squamosal has expanded to the anterior limit of the pila antotica, while the maxilla has grown posteriorly, approaching the anterior tip of the squamosal in lateral view. One small, ankylosed vomerine tooth is present on each side. A 42 mm and a 48 mm specimen show a similar degree of skull development to each other. Maxilla and palatine are fused along their entire length, except for a few larger foramina between the maxillary and palatine dental laminae. The maxillary part of the maxillopalatine has expanded posteriorly, reaching the level of the anterior margin of the pila antotica, while the squamosal has extended anteriorly beyond the anterior margin of the pila antotica. However, the orbital area lateral to the large optical foramen remains uncovered by dermal bone. In a specimen of 56 mm, and all larger ones, the squamosal has covered the orbital area and overlaps with the dorsal part of the maxillopalatine. The ossification of the anterior part of the squamosal is initially characterized by several larger foramina, which gradually decrease in size and number. Larger gaps are present between the squamosal and frontal and parietal dorsally, and maxillopalatine ventrally, but these continue to narrow, until all elements are tightly sutured by 90-100 mm total length, essentially resembling the adult condition.

From hatching on, the lower jaw is well developed and each dentary bears at least three large, ankylosed teeth. The hyobranchial skeleton appears weakly chondrified at hatching but resembles that of the adult apart from a shallow indentation between the tips of former ceratobranchials III and IV.

DISCUSSION

Adult *Boulengerula taitanus* and *Gegeneophis ramaswamii* are very similar in their overall morphology and ecology. Both species have a completely closed, stegokrotaphic skull. The eye is greatly reduced and the orbital region is completely covered by the squamosal and the maxilloplatine (Fig. 3). Both species are well adapted burrowers that spend almost their entire life under ground (Measey et al. 2004; Gower et al. 2004) and have similar adult sizes (Taylor 1968). Both species also develop directly, in that a free-living larval stage is absent. Direct development in *G. ramaswamii* and *B. taitanus* is characterized by a precocious development of the tentacle and a lack of typical larval labial folds, lateral line organs and a tail fin. The gill slit found in larvae of biphasic species is comparatively small and closes early during development, with no traces remaining at hatching. Despite these many similarities, the skulls of *B. taitanus* and *G. ramaswamii* are very different in their degree of differentiation upon hatching. Hatchling *G. ramaswamii* closely resemble adults with regard to skull shape and differentiation, but those of *B. taitanus* are much less advanced in their development and resemble embryos of *G. ramaswamii* (Müller et al. 2005) and other direct-developing species such as *Hypogeophis rostratus* (Müller 2006). These differences correlate with extended brood care provided by the mother in *B. taitanus*. After hatching, young *B. taitanus* stay with their mother and feed on her specially modified skin until they become independent, by which time their size has increased threefold (Kupfer et al. 2006; Chapter 5).

Superficially, hatchlings and young juveniles of *B. taitanus* show several similarities to larvae of the biphasic *Ichthyophis* cf. *kohtaoensis*. Both have the cheek region largely uncovered by bone, separate maxillae and palatines upon hatching, and the anterior endocranium and especially the nasal capsule is largely cartilaginous. However, larval *I.* cf. *kohtaoensis* undergo a metamorphosis somewhat like those of other amphibians – the tentacle develops and the gill slit closes, lateral line organs, labial folds and the tail fin are reduced, the larval squamosal and hyobranchial skeleton are remodelled into the adult-like shape, and the maxilla fuses to the palatine and expands rapidly posteriorly – juvenile *B.*

taitanus show none of these dramatic changes but instead gradually develop the adult-like morphology. Moreover, in hatchling *B. taitanus*, the squamosal and hyobranchial skeleton do not have a larval configuration but instead seem to assume a mid-metamorphic morphology from the beginning of ossification or chondrification, respectively. In this respect, the ontogeny of *B. taitanus* is similar to that of *G. ramaswamii*, and can be interpreted as a case of ontogenetic repatterning as commonly seen in direct developing species (Hanken 2003).

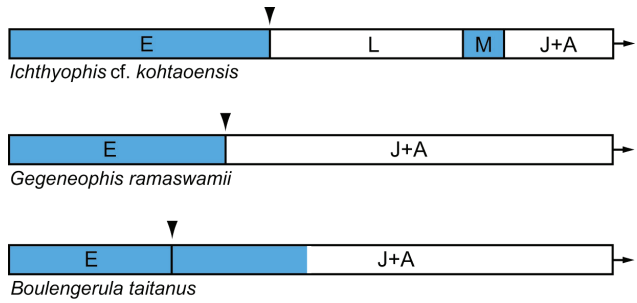

Fig. 4. Schematic representation of the life histories of *Ichthyophis* cf. *kohtaoensis*, *Gegeneophis ramaswamii* and *Boulengerula taitanus*. The arrow head marks the point where hatching occurs and blue indicates periods of morphological change. E – embryonic period; L – larval period; M – metamorphosis; J+A – juvenile and adult life.

Even though *Boulengerula taitanus* is a true direct developer like *Gegeneophis ramaswamii*, rather than possessing a curious land-larva that undergoes a postembryonic metamorphosis, the two species are nonetheless very different in their life-history and postembryonic ontogeny (Fig. 4). In *G. ramaswamii* the whole morphological development is condensed into the embryonic period (here defined as the time spent inside the egg), with very little morphological development occurring after hatching, apart from growth. In contrast, morphological development continues during most of the postembryonic care period in *B. taitanus*. However, the difference between *B. taitanus* and *G. ramaswamii* does not solely seem to be attributable to a shift in hatching time. Not only do *B. taitanus* hatch at a much smaller size than *G. ramaswamii* (~30 mm vs. ~55 mm) but the postembryonic developmental phase also seems to be prolonged. Juvenile *B. taitanus* are larger than those of *G. ramaswamii* (~80-90

mm vs. ~55 mm) by the time they have attained the same degree of skull development, with tight sutures between the squamosal and adjacent bones. This is directly correlated with the time spend under maternal care and the specialized feeding (Kupfer et al. 2006). At hatching, juvenile *B. taitanus* have a well-developed premaxilla that carries large teeth, whereas the other tooth bearing bones are smaller and carry either no teeth or much smaller ones than the premaxilla. Too little information is available on the embryonic development of these bones for a thorough analysis, but it seems nonetheless as if the development of the premaxilla in *B. taitanus* is somewhat accelerated compared to *G. ramaswamii* (Müller et al. 2005) and also *Hypogeophis rostratus* (Müller 2006). It is tempting to attribute this, together with the large teeth that show a specialized morphology (Kupfer et al. 2006), to the skin feeding seen in the juveniles. While the premaxilla (or premaxillary part of the nasopremaxilla) decreases in relative size compared to the other tooth bearing bones during further development, it remains relatively larger in *B. taitanus* as compared to *G. ramswamii* throughout the entire ontogeny (see Fig. 3B,C), which accounts for one of the most obvious differences between the adult skulls of both species.

In sum, the direct developing *Boulengerula taitanus* and *Gegeneophis ramaswamii* show ontogenetic repatterning and heterochronic shifts in their ontogeny, as compared to that of the ancestral biphasic ontogeny. It is currently unclear how the different developmental patterns observed in *B. taitanus* and *G. ramaswamii* relate to each other. The two species are only distantly related (Fig. 1 and Fig. 5) and little detailed information is available on the ontogeny of other direct developing caecilians other than *Hypogeophis rostratus* (Müller 2006) or indeed any other caecilians (see Wake 2003). At the moment, it is unclear if the degree of postembryonic skull development seen in *B. taitanus* is a special adaptation of this taxon or merely plesiomorphic, though the former hypothesis seems more plausible.

Evolution of direct development in caecilian amphibians

Several studies have recently investigated caecilian intrarelationships (Wilkinson et al. 2003; Frost et al. 2006; Roelants et al. 2007) with sufficiently dense taxon sampling to enable a preliminary discussion of the evolution of direct development in caecilians. All studies recovered a number of identical clades but differ in the relationships of these clades to each other, which complicates a consistent reconstruction of the evolution of reproductive modes. What is clear, however, is that the vast majority of 'higher caecilians' ("Caeciliidae", Scolecomorphidae and Typhlonectidae) are either known or thought to be direct developing or viviparous (Fig. 5; Wilkinson and Nussbaum 1998).

For the phylogeny of Wilkinson et al. (2003), parsimony optimization of the three main reproductive modes mapped onto the phylogeny unequivocally reconstructs direct development for the common ancestor of higher caecilians. *Herpele squalostoma*, the sister taxon to *Boulengerula*, is also reconstructed as direct developing. Viviparity evolved three times independently from a direct developing ancestor in *Scolecomorphus*, *Typhlonectes natans*, and the clade comprising *Geotrypetes seraphinii*, *Dermophis mexicanus* and *Schistometopum*. It further implies that a larva has re-evolved at least once among higher caecilians, within the Seychellean radiation, and possibly as many as three times (Fig. 5). Alternatively, assuming a free-living larva as the ancestral condition for higher caecilians requires two extra steps explaining the distribution of life-history modes on the given phylogeny. Based on the phylogeny of Frost et al. (2006), however, the reconstruction of the developmental mode of the last common ancestor of higher caecilians is ambiguous. According to this phylogenetic hypothesis, direct development, like viviparity, might have evolved only once or at least as many as three times. The reconstruction is further complicated by the unclear developmental mode of *Crotaphatrema tchabalmbaboensis*. When treating biphasic, direct developing and viviparous as ordered character states, however, the last common ancestor of higher caecilians is reconstructed as direct developing.

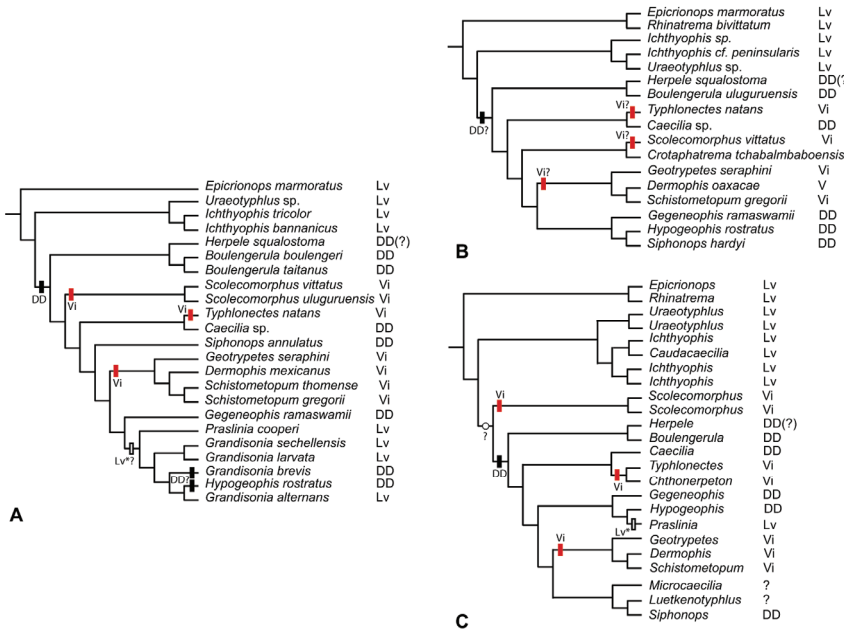

Fig. 5. Recent hypotheses of caecilian intrarelation ships. (A) from Wilkinson et al. (2003), (B) from Frost et al. (2006) and (C) from Roelants et al 2007. For each tree, the most-parsimonious character optimization is indicated, but in some cases other, equally parsimonious optimizations are also possible. DD – direct development, Lv – biphasic with a free-living larvae, Lv* – re-evolved free-living larva, Vi – viviparity, ? – indicates the possibility of alternative optimizations.

For the phylogeny of Roelants et al. (2007), the ancestral state of the last common ancestor of higher caecilians is also ambiguous for unordered character states, primarily because the viviparous *Scolecomorphus* clade is sister to all other higher caecilians. Direct development, however, is unequivocally reconstructed for the last common ancestor of the remaining higher caecilians and may have evolved only once within the group, with the possibility of a re-evolution within the Seychellean clade. Most parsimoniously, viviparity evolved three times independently, at least twice from a direct developing ancestor, and a free-living larva re-evolved from a direct developing ancestor on one occasion. *Herpele*, *Microcaecilia* and *Luetkenotyphlus* are reconstructed as being direct developing, but the developmental mode of these taxa is unknown at present (Wilkinson and Nussbaum, 1998), and the optimization changes if one or more of them are coded

as viviparous. For ordered character states, direct development is reconstructed as the ancestral condition for the last common ancestor of all higher caecilians.

The results of the ancestral state reconstructions partly demonstrate the sensitivity of the analyses to the given phylogeny and the coding of the terminal taxa and more data on both are clearly needed for a comprehensive discussion of the evolution of development in caecilians. However, even though the recent hypotheses of caecilian intrarelationships are partly incongruent, and with them the interpretations of the evolution of direct development, it seems that direct development might have evolved relatively early among higher caecilians. This interpretation is complicated by the presence of free-living larvae in some caeciliid caecilians – *Grandisonia alternans*, *G. larvata*, *G. sechellensis*, *Praslinia cooperi* (Parker 1958; Nussbaum and Gerlach 2004), and also in *Sylvacaecilia grandisonae* (Largen et al. 1972), whose phylogenetic position is unknown. Character optimization suggests the re-evolution of a free-living larva in these taxa, rather than a plesiomorphic retention. A similar reversal to a free-living larva from a direct developing ancestor has recently been proposed for some plethodontid salamanders (Chippindale et al. 2004; Mueller et al. 2004). However, caecilians are different from other amphibians in that large eggs and brood care are a characteristic of all oviparous species in the group, whether biphasic or direct developing, and were likely present in the last common ancestor of living caecilians. Although not an absolute prerequisites, direct development in frogs and salamanders is strongly positively correlated with the presence of brood care and large, yolk rich eggs (Callery et al. 2001; Nussbaum 1985). In caecilians, the evolution of direct development was probably greatly facilitated by the presence of large eggs and brood care. Modifying the ontogeny towards direct development from a biphasic, *Ichthyophis*-like ancestor seems far less challenging than altering frog ontogeny to increase egg size and delete the tadpole stage from the ontogeny. Thus, the evolution of direct development in caecilians might have been more plastic than the most parsimonious ancestral state reconstructions suggest, with possibly a repeated independent evolution in various groups within higher caecilians. Caecilians might be much better suited to

the study of the evolution of direct development than frogs or salamanders. In frogs for instance, it is difficult to dissociate changes associated specifically with direct development from those effected by an increased egg size (Callery et al. 2001). In caecilians however, biphasic and direct-developing forms share several traits such as large egg size with a presumably similar early embryogenesis and the presence of brood care, which should greatly facilitate the investigation of factors directly related to direct development. Caecilians thus have the potential to form a model comparative system for the evolution of reproductive modes in amphibian, and vertebrates in general.

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Appendix
List of specimens

Taxon	Number	Size (in mm)	Life history stage	Remarks
Boulengerula taitanus	MW03884	-	embryo	clutch of 4 embryos
	AK1003	28	hatchling	
	AK1006	30	hatchling	
	HM0036A	35	juvenile	
	HM0036B	35	juvenile	
	HM0050B	37	juvenile	
	HM0050A	42	juvenile	
	MW03912A	48	juvenile	
	MW03899	56	juvenile	
	MW03895	59	juvenile	
	MW03926	69	juvenile	
	MW03920	69	juvenile	
	MW03904	89	juvenile	
	MW03890	99	juvenile	solitary
	HM0007	117	juvenile	solitary
	MW03889	129	juvenile	solitary
	HM0042	172	subadult	
	MW03905	218	adult	
	MW3914	313	adult	
Gegeneophis ramaswamii	MW01341	42	embryo	
	MW01349	56	almost ready to hatch embryo	erroneously reported as 61mm TL in Müller et al. (2005)
	MW01280	52	hatchling	
	MW01394	56	hatchling	

	MW01063	85	juvenile
	MW00420	100	juvenile
	MW01382	101	juvenile
	MW01054	111	juvenile
	MW01587	128	juvenile
	MW01072	144	juvenile
	MW01079	165	juvenile
	MW01581	189	subadult
	MW01451	198	subadult
	MW01291	235	adult
	MW01431	240	adult
	MW01095	277	adult
	MW01560	291	adult
Ichthyophis cf. *kohtaoensis*			embryo
			hatchling
			larva
			metamorph
	MW04086		juvenile
			adult

CHAPTER 5

A novel form of parental investment by skin feeding in a caecilian amphibian

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ABSTRACT Although the initial growth and development of most multicellular animals depends on the provision of yolk, there are many varied contrivances by which animals provide additional or alternative investment in their offspring (Clutton-Brock, 1991). Providing offspring with additional nutrition should be favoured by natural selection when the consequent increased fitness of the young offsets any corresponding reduction in fecundity (Smith and Fretwell, 1974). Alternative forms of nutrition may allow parents to delay and potentially redirect their investment. Here we report a remarkable form of parental care and mechanism of parent-offspring nutrient transfer in a caecilian amphibian. *Boulengerula taitanus* is a direct developing, oviparous caecilian (Nussbaum and Hinkel, 1994), the skin of which is transformed in brooding females to provide a rich supply of nutrients for the developing offspring. Young animals are equipped with a specialised dentition, which they use to peel and eat the outer layer of their mother's modified skin. This new form of parental care provides a plausible intermediate stage in the evolution of viviparity in caecilians. At independence, offspring of viviparous and oviparous dermatotrophic caecilians are relatively large despite being provided with relatively little yolk. The specialised dentition of skin-feeding (dermatophagous) caecilians may constitute a preadaptation to the foetal feeding on the oviduct lining of viviparous caecilians.

Amphibians are renowned for their diverse forms of parental investment including hiding, guarding, transporting or feeding their offspring (Lehtinen and Nussbaum, 2003; Nussbaum, 2003) The reproductive diversity of the tropical, caecilian amphibians is more poorly known than that of salamanders and frogs, although it is known to include both oviparity, with an aquatic larva or direct development, and viviparity. Viviparous caecilians are unusual in having a specialised deciduous, foetal dentition Parker and Dunn, 1964) which is thought to be used to scrape secretions and cellular materials from the hypertrophied lining of the maternal oviduct(Parker, 1956; Taylor, 1968; Welsch et al., 1977; Wake and Dickie, 1998; Exbrayat, 2000). In contrast, it is generally thought that oviparous caecilians provision their offspring only with yolk, with additional investment limited to attendance of egg clutches (Himstedt, 1996).

In the 1990's we discovered teeth in hatchlings of the oviparous Neotropical caecilian *Siphonops annulatus* that resemble more the foetal teeth of viviparous caecilians than teeth of adults of this species (Wilkinson and Nussbaum, 1998). Field observations revealed that hatchlings are altricial and remain with their mothers until they have grown substantially. Mothers also have a paler skin colour than non-attending adults. Speculating upon these observations, we hypothesised that the foetal-like dentition of newborn *S. annulatus* is used to feed upon glandular secretions of the mother's skin (Pennisi, 1999), analogous to mammalian lactation. Here we report detailed observations of *Boulengerula taitanus*, another oviparous (Nussbaum and Hinkel, 1994) caecilian species that has altricial (Malonza and Measey, 2005) young equipped with 'foetal-like' teeth, including observations of several bouts of feeding. Rather than scraping up skin secretions, the young of *B. taitanus* use their teeth to peel and eat the specially modified skin of their mothers.

Twenty-one females of the Kenyan caecilian *Boulengerula taitanus*, with broods of between two and nine young, were collected from subterranean nest sites and maintained and observed in captivity. Eight episodes of skin feeding by different young from five different broods were observed and five filmed (see Fig. 1b, and Supplementary Movie 1 and 2). In each episode, the young moved over and around their mother's bodies, vigorously pressing their heads against their mothers while repeatedly opening and closing their mouths, and using their lower jaws in particular, to lift and peel the outer layer of the mother's skin. During one week of maternal care the young increased their total length substantially (~ 11%, Fig. 1c) with average individual growth estimated to be about 1mm per day. No alternative feeding of young was observed, and the stomachs of control young sacrificed in the field immediately upon collection contained only monolayers of skin, suggesting that ingested skin alone provides sufficient nutrients for the considerable growth observed. Maternal weight loss over the same period (~ 14%, Fig. 1c) is consistent with skin feeding imposing a high cost upon mothers.

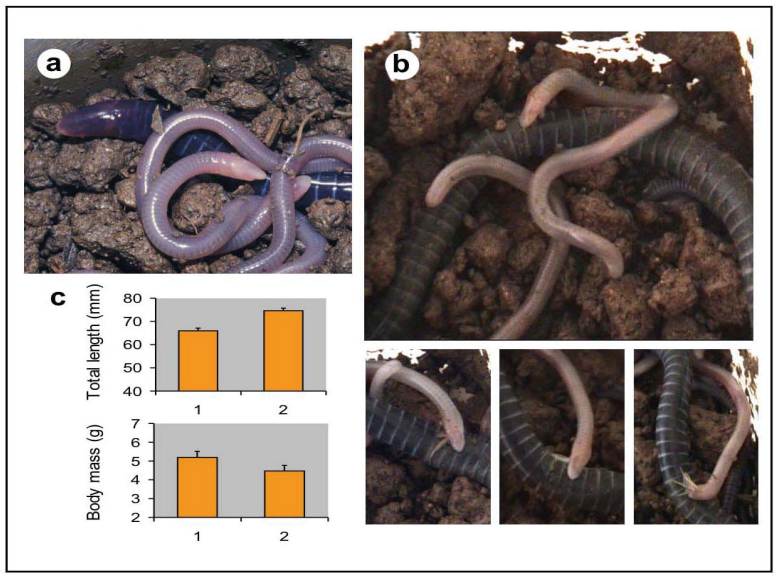

Figure 1 Skin feeding in *Boulengerula taitanus*. **a**, Female with unpigmented young. **b**, Various stills from video footage of a young peeling and eating the outermost layer of its mother's skin. **c**, Changes in mean total length (n = 66) of young (top graph) and mean body mass (n = 15) of mothers (bottom graph) between a first (1) and a second (2) measurement after one week of parental care. Bars give the standard errors of the means.

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Most attending females of *Boulengerula taitanus* were notably paler than non-attending adults, reflecting differences at cellular and tissue levels associated with the skin's novel role in nutrition (Fig. 2). The outermost layer of the epidermis, the *stratum corneum*, typically comprises squamous (flattened), keratinised cells (Fig. 2 top) whereas in brooding females the cells are far more voluminous and full of vesicles (Fig. 2 bottom). Overall, the epidermis of

brooding females is up to twice the thickness of that of non-brooding females, due to elongation of the stratified epithelial cells rather that any increase in numbers of cells. Histochemistry reveals that, unlike non-brooding females, the cytoplasm of modified epidermal cells of brooding females is full of lipid inclusions (staining positive for sudan black B). Tests for carbohydrate (alcian blue and PAS) proved negative. The *stratum corneum* is rich also in protein (staining positive for bromophenol blue) in both brooding and non-brooding females.

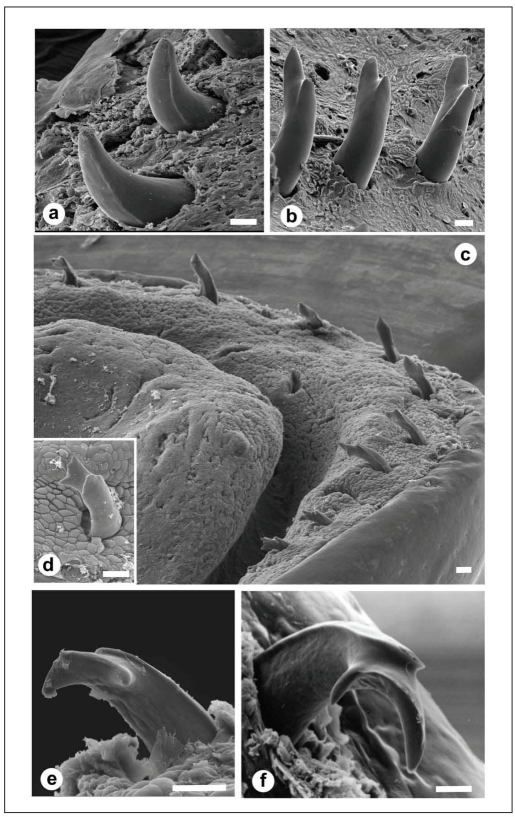

Figure 3 Dentition of adult and young *Boulengerula taitanus*. **a**, anterior view of two monocuspid, adult premaxillary teeth; **b**, labial view of three bicusped, adult vomerine teeth; **c**, lateral view of a lower jaw of a young specimen (total length 69 mm), showing different dentary tooth crown morphologies. **d**, labial view of a posterior dentary tooth of this young specimen. **e**, anterior premaxillary tooth of the same specimen. **f**, anterior premaxillary tooth of a smaller specimen (total length 57 mm) resembling a grappling hook. Scale bars, 30 μm.

Adult *Boulengerula taitanus* are predators and have two rows of pointed teeth in the upper (premaxillary-maxillary and vomeropalatine) and in the lower (dentary and splenial) jaws with either one (Fig. 3a) or two (a labial and more apical lingual) distinct cusps (Fig. 3b)(Nussbaum and Hinkel, 1994). In contrast,

tooth crowns of the dermatophagous young are remarkably variable (Fig. 3c to f). Bicusped splenial teeth are present but not yet erupted. The vomeropalatine teeth and the anteriormost three or four teeth of the premaxillary-maxillary and the dentary series are monocuspid. The remaining teeth are multicusped, combining a pronounced blade-like labial cusp with a lingual cusp that has two or three subsidiary cusps (Fig. 3d) which may be short and blunt (Fig. 3e) or more elongate and pointed processes resembling a grappling hook (Fig. 3f). Hatchling *B. taitanus* (~ 28 mm) have several other unusual characteristics, seemingly associated with their altriciality. The skull and axial skeleton are in an embryonic state of development as compared to other direct developing species (Müller et al., 2005), and the body musculature and associated external annulation are weakly developed, severely constraining mobility. By the time they become independent of their mothers (~ 86 mm), young resemble miniature adults in these features.

Dermatotrophy, as seen in *Boulengerula taitanus*, is a highly unusual mode of parental care previously unknown in tetrapods, in which nutrient provisioning involves remarkable adaptations of both the mothers and the young. Many vertebrates periodically shed their *stratum corneum* and some eat and recycle nutrients from their own shed skin (autodermatophagy)(Weldon et al., 1993). In contrast, the altricial young of *B. taitanus* depend for a time entirely upon their mother's skin, which is suitably transformed to provide nutrient that, like mammalian milk, is rich in lipids. Amphibian skin is well known for its diverse functions (Toledo and Jared, 1993, 1995) and its novel role in *B. taitanus* can be presumed to impose constraints upon other normal functions. For example, dermal granular glands are frequently associated with toxic secretions with a defensive function in amphibians (Toledo and Jared, 1995), and some down-regulation of toxins during skin feeding might be expected. Aggressive dermatophagy could injure the mother, and we might also expect the periodic bouts of feeding to be more or less synchronised with the maternal sloughing cycle, which may itself be modified, and to involve some signalling between

parent and offspring. There is clearly scope for both parent-offspring conflict and sibling competition where there is dermatotrophic parental care.

Oviparous caecilians were previously believed to only guard their eggs until hatching and to provide no subsequent parental care (Taylor, 1968; Himstedt, 1996), as in *Ichthyophis* (Kupfer et al., 2004). This nutritional investment in offspring only in the form of yolk (lecithotrophy) is seen in all 'primitive' caecilians and is inferred to be the ancestral condition, with viviparity, and foetal feeding on the maternal oviduct lining (matrotrophy), being derived. Maternal dermatotrophy provides a highly plausible intermediate between these different reproductive modes. Current understanding of caecilian phylogeny (Wilkinson et al., 2003) indicates that viviparity must have evolved independently several times in caecilians, which implies striking and enigmatic convergent evolution of the associated foetal teeth. The discovery of foetal-like teeth in maternal dermatotrophic caecilians suggests that although viviparity is convergent in caecilians, one of its most distinctive features, foetal teeth, might not be. Foetal-like teeth, are known also in some species of the oviparous Neotropical genera *Siphonops* and *Caecilia* (Parker and Dunn, 1964; Wilkinson and Nussbaum, 1998), and the distribution of foetal and foetal-like teeth across viviparous and oviparous caecilians is consistent with their having a single origin and thus being homologous (see Supplementary Information). This implies that the independently derived lineages of viviparous caecilians evolved from (possibly maternal dermatotrophic) ancestors that already possessed a specialised dentition which was preadapted to feeding in oviducts. This reconstruction and current estimates of divergence times (San Mauro et al., 2005) suggests that foetal-like teeth evolved in the Mesozoic and that some form of skin feeding may have persisted in caecilians for at least 150 million years.

The use of foetal-like teeth in other oviparous caecilians that possess them, whether in maternal dermatotrophy as exemplified by *Boulengerula taitanus*, or some other kind of feeding has not yet been documented. Newborns of the viviparous, West African caecilian genus *Geotrypetes* are altricial and it has been speculated, but not demonstrated, that they may feed on the maternal

skin or its secretions (O'Reilly et al., 1998; Pennisi, 1999). A single reported newborn of the viviparous East African caecilian genus *Scolecomorphus* has a peculiar oral morphology that may be associated with specialised feeding after parturition (Loader et al., 2003). Careful observation of these and other as yet unstudied caecilians may reveal additional forms of parental care that are plausible intermediates between, or otherwise help explain, the major evolutionary transitions in caecilian reproduction.

One potential advantage to feeding young rather than providing them with yolk alone, is that investment can be delayed, and if advantageous, redirected. Both maternal dermatotrophic and viviparous caecilians produce relatively fewer, larger independent offspring than lecithotrophic caecilians (AK, unpublished). Selection for larger offspring is hypothesised to have driven the evolution of extended parental care in salamanders (Nussbaum, 2003) and may have similarly driven the evolution of the peculiar derived life histories in caecilians.

In recent years, the known species diversity of amphibians has been steadily increasing mainly as a result of biodiversity surveys in the tropics (Haddad and Prado, 2005). At the same time there has been growing concern about apparently declining amphibian populations world-wide. Recently the Global Amphibian Assessment identified many data deficient species (20%) and the urgent need for more information (Gower and Wilkinson, 2005; Stuart et al., 2005). Our discovery underscores the need for further studies to better document the amazing diversity of amphibian life history strategies and greater efforts to conserve it.

Methods

We studied the caecilian *Boulengerula taitanus* in the field in South-eastern Kenya (Wundanyi, Taita hills, Taita -Taveta Destrict). Most fieldwork was carried out after the short rainy season (Vuli), in January of 2004 and 2005 following preliminary fieldwork in January 1996. Field-collected females and their young were housed in small plastic boxes (9x9x3.5cm) containing earth

moulded to resemble nests observed in the field. Observations were made daily from 6 am to 9 am and from 8pm to midnight. Behaviours were recorded with a digital-video-camera (Sony DCR-HC40E). The total length of young during parental care was measured on a plastic-coated mm-sheet.

Skin tissue of brooding and non-brooding females was fixed in buffered formalin and/or Bouin's. Samples were embedded following standard procedures (Romeis, 1989). Paraffin sections (6-8µm) were cut with a rotary microtome and stained with either haematoxilin/eosin, sudan black B, bromophenol blue, alcian blue pH 2.5 or PAS.

Tooth morphology of young and adults was analysed with a scanning electron microscope (Hitachi 2500 series). Samples were transferred through an acetone series and critical point dried using carbon-dioxide, mounted on aluminium stubs, and sputter coated with gold-palladium.

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Supplementary material

Supplementary Video 1

Excerpt of a real time video clip of skin feeding in *Boulengerula taitanus* taken on January 21, 2005. Dermatotrophy is otherwise unknown in tetrapods but a form of skin feeding occurs in some cichlid fishes (e.g. *Symphysodon aequifasciatus*) where young feed upon the proliferated multilayered epidermis of both parents (Bremer, 1999), rather than peeling a specialised monolayer of the maternal epidermis.

Supplementary Video 2

Excerpt of a real time video clip of skin feeding in *Boulengerula taitanus* taken on January 21, 2005. Stills (video captures) from this sequence appear in Fig. 1b of the text.

Both videos are available at:
http://www.nature.com/nature/journal/v440/n7086/suppinfo/nature04403.html

Supplementary Figure 1

Distribution, and parsimonious interpretation of the evolution of, viviparity and of modified foetal or foetal like teeth. Reconstruction uses the maximum likelihood tree recovered in the most recent broad phylogenetic analysis of caecilian interrelationships using molecular sequence data (Bremer, 1999). At least three independent origins are needed to account for the observed distribution of viviparity (defined here as young hatching before eggs are laid) in caecilians, whereas a single origin accounts for the presence of foetal and foetal like teeth in the viviparous and oviparous caecilians that have them.

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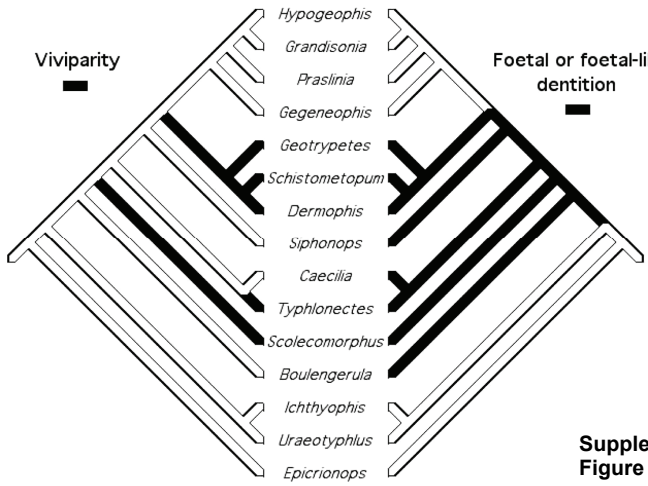

Supplementary Figure 1

CHAPTER 6

Morphology and function of the head in foetal and juvenile *Scolecomorphus kirkii* (Amphibia: Gymnophiona: Scolecomorphidae)

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ABSTRACT We describe the external and musculoskeletal morphology of the head in an ontogenetic series of the scolecomorphid caecilian *Scolecomorphus kirkii*. Juvenile specimens show a similarly unusual morphology as described by Loader et al. (2003) for a single juvenile specimen of *S. vittatus*. The rostral region is expanded into large, posterolaterally pointing paraoral processes that are formed by the maxilla. Extraoral teeth that show signs of wear are present on the underside of the rostrum in front of the mouth and laterally on the paraoral processes. Foetuses are characterized by a similar morphology but the teeth are covered by epidermal tissue. The endoskeletal part of the skull is largely cartilaginous, while all of the dermal bones, with the exception of the squamosal, are well developed. The foetal chondrocranium is extensively developed and shows a peculiar posteriorly directed process of the lamina orbitonasalis posterolaterally, which is joined by a transverse bar joining the pila preoptica posterior to the choana, and a posteriorly directed lateral process (postpalatine process) that extends parallel to the trabecular cartilage beyond the level of the posterior end of the pila antotica. Only two primary jaw adductor muscles are present, together with two pterygoideus-like muscles that insert onto the lower jaw. The palatoquadrate, respectively quadrate of foetuses and juveniles shows a high degree of mobility. The possible function of this unusual skull morphology is discussed and it is suggested that is an adaptation to post parturition feeding.

Introduction

Caecilians are elongated, limbless amphibians mostly inhabiting soils in parts of the wet and seasonal tropics and subtropics (Wilkinson and Nussbaum, 2006). Because of their superficially snake-like appearance and a general paucity of external characters, caecilians are often considered to be a uniform group with only minor modifications of the common ground pattern (Himstedt, 1996). This view however, is increasingly challenged by recent discoveries of remarkable specialisations of individual taxa including, among others, novel modifications of the cardiovascular system in the caeciliid *Herpele squalostoma* (Wilkinson, 1992)

and lunglessness with many associated radical morphological changes in the typhlonectid *Atretochoana eiselti* (Nussbaum and Wilkinson, 1995).

Caecilians show a remarkably rich and increasingly appreciated diversity of early life-histories. Although a small group of only about 170 currently recognized species (Wilkinson and Nussbaum, 2006), caecilians exhibit all main reproductive modes found in other amphibians: oviparity with a free-living aquatic larva, oviparity with direct development, and viviparity. All oviparous caecilians, as far as is known, provide brood care in that females guard their clutches until hatching (e.g. Goeldi, 1899; Sanderson, 1937; Kupfer et al., 2004). Viviparous forms have developed various forms of intraoviductal nutrient transfer, either via oviductal secretions and hypertrophied oviductal epithelium, which is scraped by the foetuses equipped with a specialized foetal dentition (Parker, 1956; Wake, 1977), or via modified embryonic gills that function analogous to a placenta (Delsol et al., 1986; Exbrayat and Hraoui-Bloquet, 1992). Kupfer et al. (2006; Chapter 5) described a novel form of parental care in the direct-developing caeciliid *Boulengerula taitanus*, where the young feed on their mother's skin, which is specially modified during a period of post hatching care. A similar form of parental care has also been postulated for the viviparous caecilian *Geotrypetes seraphini*, which gives birth to small, precocious young that might be nourished by skin secretions of the mother (O'Reilly et al., 1998; see also Pennisi, 1999).

Recently, Loader et al. (2003) described a morphologically remarkable juvenile of *Scolecomorphus vittatus*, from the North Pare Mountains of Tanzania. This specimen is characterized by conspicuous, posteroventrally directed paraoral processes that bear teeth on their aboral sides, an unusually short lower jaw and other features previously unknown of any life history stage of any caecilian. Loader et al. (2003) suggested that this highly divergent juvenile morphology might be indicative of a specialized life-history stage. *Scolecomorphus vittatus* belongs to the Scolecomorphidae, a little known family of African caecilians that comprises the genera *Crotaphatrema* and *Scolecomorphus*, which occur with three species each in West and East Africa, respectively (Taylor, 1969a;

Nussbaum, 1985; Lawson, 2000). Scolecomorphids have several morphological characteristics unique among caecilians, such as a completely covered oval fenestra and no stapes (de Villiers, 1938), eyes that protrude with the extrusion of the tentacle (O'Reilly et al. 1996) and an unusually kinetic skull (Trueb 1993).

Here we describe the external morphology of foetuses and juveniles of *S. kirkii* from several well-preserved specimens from the Udzungwa Mountains of Tanzania. We furthermore provide the first description of the morphology of the skull and lower jaw and their associated musculature in foetuses and juveniles based on computerized 3D reconstructions from serial sections and cleared and stained specimens, and briefly discuss functional implications.

Material and Methods

We studied an ontogenetic series consisting of foetuses, juveniles, sub-adults and adults of *Scolecomorphus kirkii* from the West Kilombero Scarp Forest (BMNH) and the Njokamoni River drainage, Udzungwa Mountains National Park (AMNH), Udzungwa Mountains, Tanzania (for a complete list of specimens see Appendix). As juveniles we classified those animals that exhibited the same unusual morphology as described by Loader et al (2003) for *S. vittatus*, while larger specimens with an adult-like morphology are regarded as sub-adults. Animals were collected in the field and either fixed in formalin or ethanol and subsequently stored in ethanol. Nomenclature cranial musculature follows Kleinteich and Haas (2007).

Specimen preparation and investigation

Specimens selected for clearing and staining were double stained for bone and cartilage using a slightly modified protocol based on Taylor and Van Dyke (1985), and dissected for musculature before applying the final steps of the protocol. Where necessary, musculature was stained using the method of Bock and Shear (1972). Gross dissections and drawings were made with the aid of a Nikon SMZ-U stereomicroscope equipped with a camera lucida. Specimens selected for histology were processed following standard procedures, serially

sectioned transversely at 8 µm, and variously stained with Haematoxylin and Eosin, Haematoxylin and Masson's Trichrome or Mallory's Phosphotungstic Acid Haematoxylin (Böck 1989). One foetus was selected for scanning electron microscopy, dehydrated, critical point dried, sputter-coated with gold-palladium and examined using a Hitachi 2500 SEM.

3D reconstruction

For the computerized 3D-reconstruction, every third histological section was digitized using a Leica BD5000 microscope with a digital image capture system. The resulting images were aligned using the programme Autoaligner (Biplane AG, Switzerland) and the correct alignment verified by subsequent inspection of the image stack and manually corrected where necessary. For the 3D-reconstructions, the image stack was imported into the programme Imaris 4.0.5 (Bitplane AG, Switzerland). A scene was created in the program module 'Surpass' and the contours of the studied elements were marked manually on every section with a polygon. All relevant structures were reconstructed separately, then combined and subsequently rendered to produce the final images. Some of the developing dermal bones including the frontal, parietal and especially parasphenoid showed a reticulated growth pattern, with numerous bone trabeculae and small foramina at their leading edge. A detailed reconstruction of such bone patterns is not feasible from serial sections and these were reconstructed as sold plates instead. Teeth were not reconstructed.

Results

All foetuses are in a similarly advanced state of development. No yolk is visible externally, except for the moderately enlarged intestine that is still visible through the ventral side and apparently filled with yolk. Three long gills are present laterally behind the head on each side, the second gill being the longest followed by the first and the third. All gills bear numerous gill filaments. No gill openings are discernible. The head appears quite broad in dorsal view, with blunt, laterally projecting paraoral processes (Fig. 1). The tentacles are in a lateral position, on a

line from the upper corner of the mouth to the nostril, and the rim of the tentacular aperture is visible dorsally. Somewhat darker pigmented eyes are positioned above and slightly behind the base of the tentacle but only faintly visible. In lateral view, the head is triangular to wedge- shaped. The underside of the rostrum is almost completely flat and has a triangular shape when viewed ventrally. On the underside of the rostrum, approximately 0.5 mm from the margin of the mouth, is a parallel line of around eight knob-like protuberances (Fig. 1, Fig. 2). On each side, one or two additional protuberances are present posterolaterally on the paraoral process. At high magnification, the tip of a tooth can be seen shining through the epidermis of each protuberance. The upper margin of the mouth and, correspondingly, the lower jaw are very broadly rounded. When pressed, the lower jaw fits the upper margin of the mouth closely. The lower jaw bears protuberances on its anterior side, similar to those seen on the underside of the rostrum, but smaller and more numerous. These protuberances represent epidermis-covered dentary teeth and are arranged in two to three rows, three around the symphysis and two more laterally. All foetuses have a weakly developed band of dark pigmentation that covers the dorsal and dorsolateral sides of the body and stretches from the tip of the snout to the body terminus, excluding the nostrils, tentacles and the paraoral processes. Three juvenile specimens showing the morphology described by Loader et al. (2003) for a juvenile *S. vittatus* were available for study. These specimens are 93 mm, 104 mm and 106 mm in total length. All are of similar appearance and have a well-developed band of dark pigmentation that covers the dorsal and dorsolateral sides of the body and stretches from the tip of the snout to the body terminus, similar to the adult colouration (Fig 1). The areas around the nostrils, the bases of the tentacles and the paraoral processes are free of pigmentation. The tentacles are visible dorsally in all specimens, although to varying degrees. The ventral side of the rostrum is markedly convex transversely. Teeth are visible in the positions corresponding to the protuberances seen in the foetuses and are part of the premaxillary-maxillary series. All teeth are relatively large, straight and bicuspid, with the accessory cusp very small and apically positioned. An additional one to

two teeth are found on the lateral side of the paraoral processes, pointing laterally, posterolaterally or dorsolaterally. The teeth found on the paraoral processes and those laterally on the underside of the rostrum show clear signs of wear.

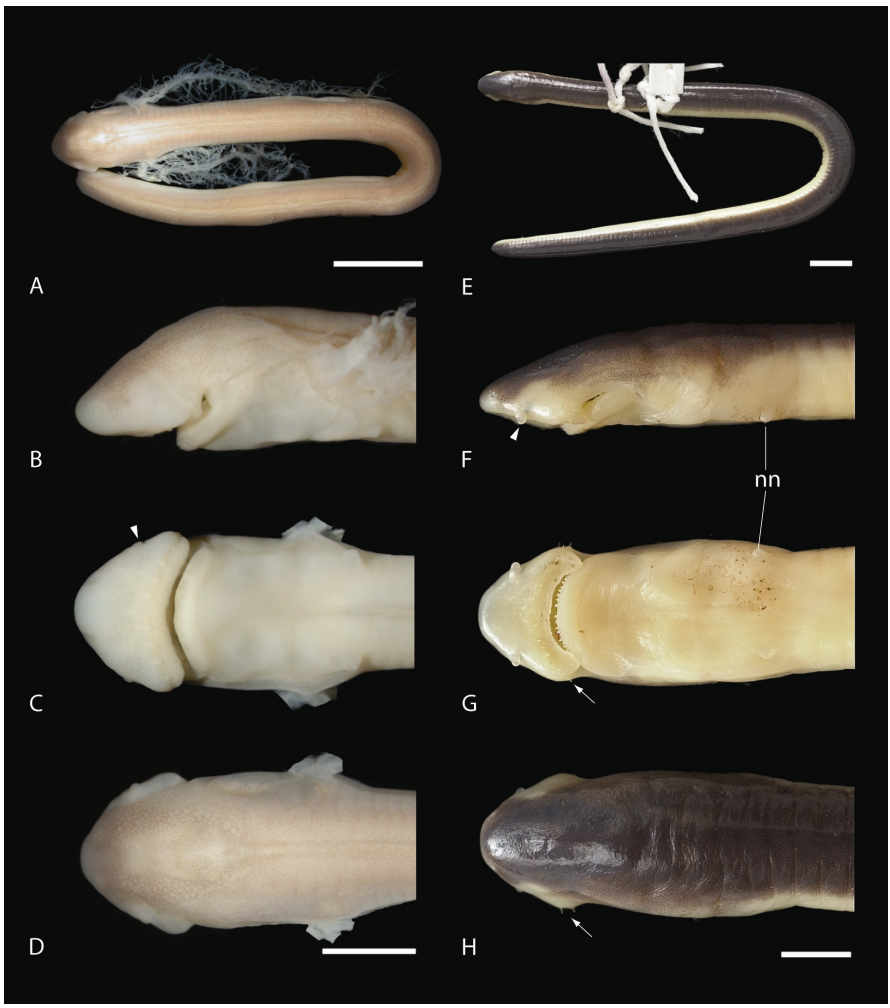

Fig. 1. A Total dorsal view of a foetal *Scolecomorphus kirkii* and a close-up of the head in lateral (B), ventral (C), and dorsal (D) view. Note the long external gills in A; arrow head marks the tentacle anlage in C. E Total dorsal view of a juvenile *Scolecomorphus kirkii* and a close-up of the head in lateral (F), ventral (G), and dorsal (H) view. Arrow head points to tentacle in F, also note the eye seen as a dark spot at the base of the tentacle. Arrows point to extraoral teeth on the lateral sides of the paraoral process. nn – nuchal nipples. Scale bars equal 5 mm in A and E and 2 mm for all remaining.

This is more pronounced in the two larger specimens, where some of the teeth on the outside of the paraoral processes are worn down to almost the level of the skin, than in the smaller juvenile. The teeth on the lower jaw are also erupted and are arranged in three to four rows, with four rows confined to the symphyseal region. Towards the jaw articulation, teeth are large and monocusped and arranged in a single row. The other teeth in multiple rows are lancet-shaped. The largest of these are the inner most with the outer rows being successively smaller. The outer rows of teeth also show heavy wear, where tooth crowns are worn down considerably. This is again more so the case in the larger juveniles than in the smallest. The posterior part of the gut is completely filled by a whitish, amorphous mass that superficially resembles yolk. A Haematoxylin and Eosin stained smear revealed it to be composed of numerous small spheres, some cellular debris and isolated nuclei, and a few soil particles.

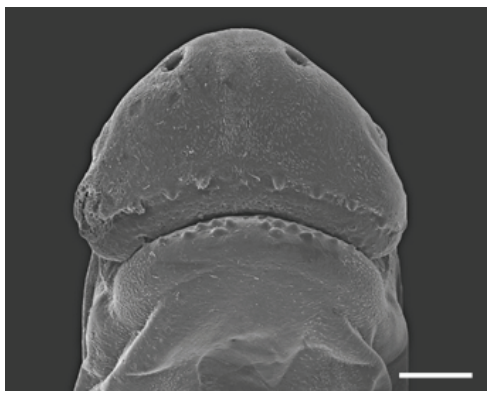

Fig. 2. SEM photograph of the ventral side of the head of a foetal *Scolecomorphus kirkii*. Note the epidermis covered teeth on the upper and lower jaw. Scale bar equals 0.5 mm.

Musculoskeletal morphology The endocranium of the foetuses is almost completely cartilaginous (Fig. 3). The only endocranial ossifications present are the exoccipital in the upper skull and, in the lower jaw, the retroarticular process and the perichondral ossifications surrounding the symphysis. The nasal capsule is very prominently developed. Most of the floor of the anterior capsule is cartilaginous except for a relatively small prechoanal foramen and a smaller foramen for a ventral branch of the ophtalmicus profundus nerve, medial to the prechoanal foramen. Further caudally is a very large choanal foramen that is bordered by the trabecular plate and pila preoptica medially, the solum nasi

anteriorly and anterolaterally, and a posteriorly directed process of the lamina orbitonasalis posterolaterally. The posterior border of the choanal foramen is

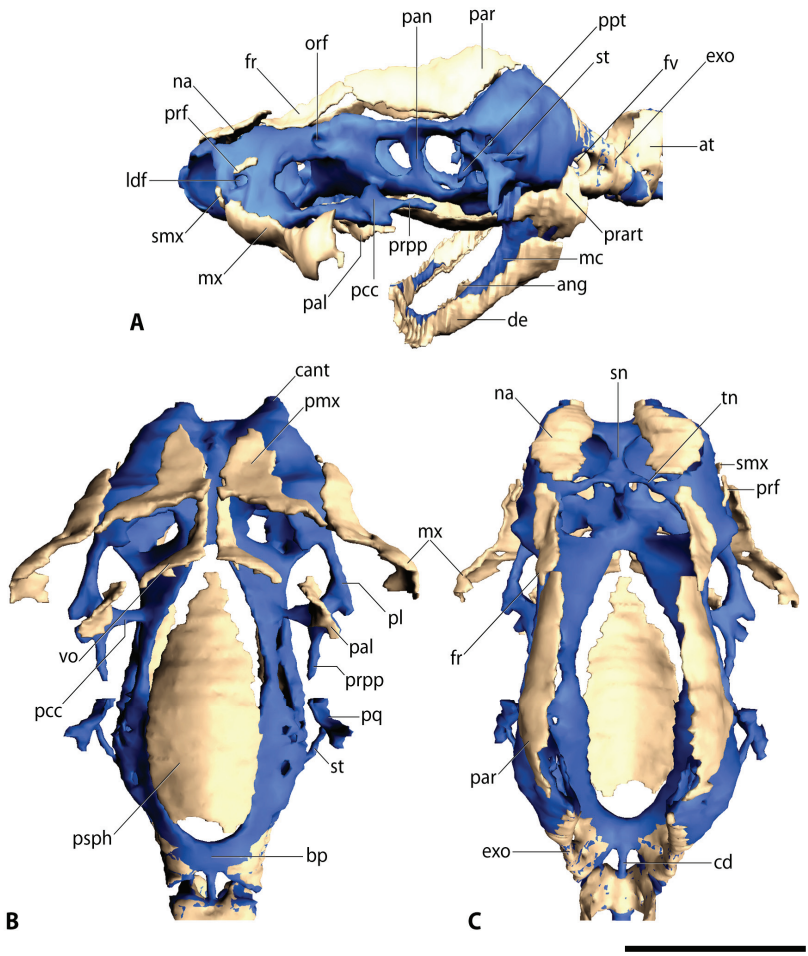

Fig. 3. Three dimensional reconstruction of the skull of a foetal *Scolecomorphus kirkii* in (A) lateral, (B) ventral and (C) dorsal view. Lower jaw omitted in B and C. at, atlas; bp, basal plate; cant, anterior copula; cd, chorda dorsalis; de, dentary; exo, exoccipital; fr, frontal; fv, vagus foramen; mc, Meckel's cartilage; mx, maxilla; na, nasal; orf, orbitonasal foramen; pal, palatine; pan, pila antotica; par, parietal; pcc, postchoanal commissure; pmx, premaxilla; ppt, pterygoid process of the palatoquadrate; pq, palatoquadrate; prart, retroarticular process; prf, prefrontal; prpp, postpalatinal process; psang, pseudoangular; psph, parasphenoid; smx, septomaxilla; sn, septum nasi; st, stapes; tdf, tentacular duct foramen; tm, taenia marginalis; tn, tectum nasi; vo, vomer. Scale bar equals 1 mm.

formed by a transverse bar joining the pila preoptica and the posteriorly directed lateral process of the lamina orbitonasalis. Posterior to this commissure, the lateral process extends parallel to the trabecular cartilage beyond the level of the posterior end of the pila antotica. This posteriorly directed process is provisionally termed the postpalatinal process here, in reference to its position posterior of the initial position of the palatine, while the transverse bar is provisionally termed the postchoanal commissure. The lateral wall of the nasal capsule is also completely chondrified but has a foramen for the passage of the tentacular ducts (homologous to the nasolacrimal ducts [Sarasin & Sarasin, 1887-1890]). The dorsal aspect of the capsule is characterized by a large foramen that is bordered anteriorly by the slender copula anterior, laterally by the cartilago obliqua, medially by the septum nasi and posteriorly by a slender tectum nasi. The medial part of the nasal capsule is formed by a relatively simple nasal septum. A prenasal process is absent. Posteroventrally to the nasal septum, between the anterior parts of the choanal foramina, is a broad trabecular plate that is formed by the fusion of the trabecular cartilages. The notochord extends anteriorly onto the basal plate but does not project into the basicranial fenestra.

With the exception of the squamosal, all dermal bones found in the adult skull are already present in the foetus. The premaxilla consist of a well-defined dental lamina that spans almost the entire width of the nasal capsule, and a broad, triangular alary process that covers the ventromedial part of the anterior half of the nasal capsule. The maxilla lies lateral to the premaxilla and extends from just rostral and underneath the prechoanal foramen posteriorly and covers the ventrolateral side of the posterior half of the nasal capsule. At the level of the posterolateral tip of the premaxilla, the maxilla bends posterolaterally, extending into the paraoral process. A foramen for the maxillary nerve is present where the maxilla starts bending outward. The lateral process of the maxilla becomes increasingly concave towards its posterior end and attains a c- or u-shape in transverse sections. Immediately anterodorsally to the maxilla is a small, plate-like septomaxilla. The prefrontal is also rather small, about twice as big as the septomaxilla, and has the form of a narrow plate that extends posteriodorsally

from above the tentacular duct foramen. The nasal is relatively broad and covers the dorsolateral side of the nasal capsule. Both nasals are widely separated by a gap approximately the width of a single nasal. Posterior to the nasal, the frontal covers the dorsolateral side of the anterior part of the brain. It is followed immediately posterior by the parietal, which covers most of the dorsolateral side of the brain not covered by the frontal. Both frontal and parietal have a reticulated leading edge, with numerous small foramina and other ossification deficiencies, as seen in the cleared and stained specimen. Only the frontal and parietal overlap slightly. The vomer consists of a dental lamina and a conspicuous, slender, anteriorly directed process that extends underneath the premaxilla. The dental lamina has two dorsally directed processes that enclose the palatine branch of the facial nerve. Lateral to the posterior end of the vomer is the small palatine, which rests on the posterolateral process of the lamina orbitonasalis, at the level of the commissure of the process with the pila preoptica. The palatine has an intimate connection with the chondrocranium and is moulded around it. The basicranial fenestra is almost completely filled by the plate-like parasphenoid, except for an anterior medial and lateral gap and a smaller posterior medial gap just anterior of the basal plate.

Only two primary jaw adductors are present. The *m. adductor mandibulae articularis* is the smaller of these and originates from the anteromedial side of the palatoquadrate and inserts on the dorsal side of the lower jaw immediately in front of the jaw articulation. The much larger *m. adductor mandibulae longus* originates from the lateral side of the parietal and the taenia marginales, and inserts on the dorsal side of the lower jaw, in front of the *m. adductor mandibulae articularis* insertion. The two muscles are separated by the mandibular branch of the trigeminal nerve. Lateral to these muscles is the *m. depressor mandibulae*, which originates from the fascia covering the *m. add. mand. longus*, the parietal and dorsal otic capsule, and inserts onto the dorsomedial side of the retroarticular process of the lower jaw. It covers the dorsal half of the *m. add. mand. longus* and the otic capsule. Medial to the lower jaw, two trigeminus innervated muscles are present. A large *m. pterygoideus*

originates from the ventral side of the otic capsule and attaches to the medial side of the retroarticular process. Anterior to the *m. pterygoideus* is a smaller muscle that originates from the postpalatine process via a tendon and also attaches to the medial side of the lower jaw, close to the jaw articulation. Although both muscles run parallel along the lower jaw, both are completely separated and have a different fibre orientation, with the fibres of the *m. pterygoideus* being more oblique while those of the smaller muscles run almost parallel to the lower jaw. The fan-like *m. intermandibularis* originates from the medial side of the pseudoangular, anterior of the jaw articulation and inserts in a mid-ventral fascia. It slightly overlaps the *m. interhyoideus* at its posterior end. The facialis innervated *m. interhyoideus posterior* has an anterior slip that is slightly narrower than the *m. intermandibularis* and originates from the ventral edge of the retroarticular process and inserts in the mid-ventral fascia. A larger, posterior slip of the *m. interhyoideus posterior* originates from the lateral and ventral edge of the retroarticular process and inserts in the mid-ventral fascia ventrally and the fascia overlying the epaxial and hypaxial musculature. The ventral-most fibres of the *m. interhyoideus posterior* have a more anterolateral attachment on the lower jaw, very close to the jaw articulation and in line with the anterior limit of the articular facets. Posteriorly, this muscle fans out dorsally behind the gill attachment site.

The ossification of the smallest juvenile is much advanced compared to the foetus, although it shows essentially the same morphology (Fig. 4). Most of the endocranium is well ossified apart from parts of the nasal capsule (the copula anterior, parts of the solum nasi and cartilago obliqua). These nasal capsule components are reduced in extent compared to the foetus, except for the copula anterior. Most of the anterior endocranium is incorporated into the sphenethmoid ossification. However, the commissure between the pila preoptica and the posterolateral process of the lamina orbitonasalis remains cartilaginous and seems to buttress the maxillopalatine against the sphenethmoid. Most of the peculiar posterolateral and caudal process has disappeared although some of it seems to have been incorporated into the maxillopalatine (see below). Between the

sphenethmoid and the os basale, two blocks of cartilage remain dorsal and ventral to the optic foramen. The posterior part of the endocranium has fused with the

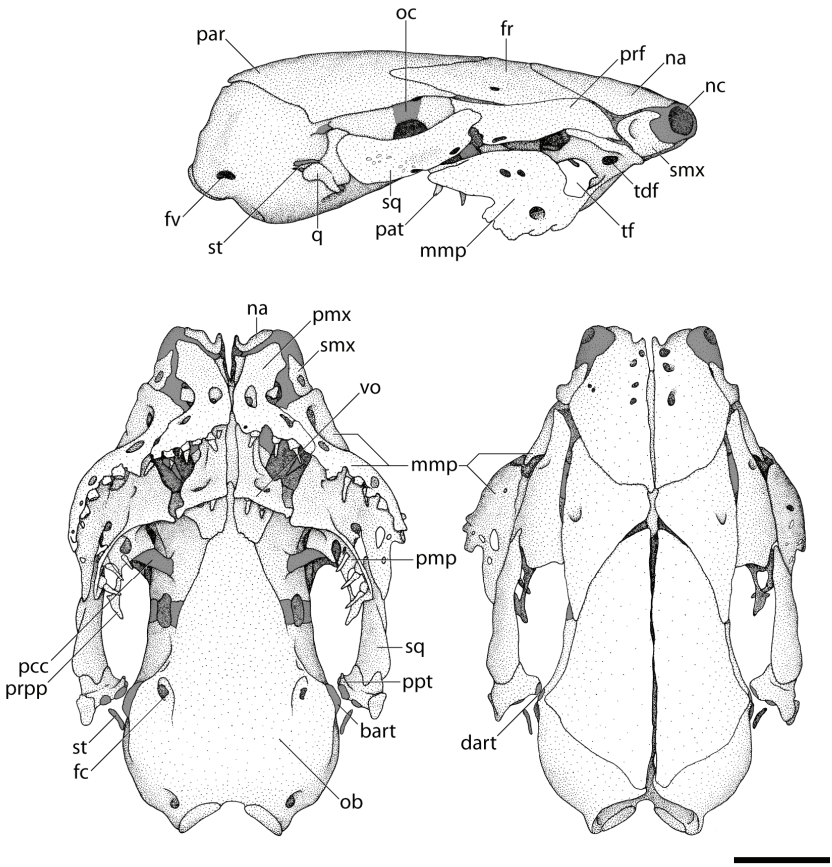

Fig. 4. Juvenile skull of *Scolecomorphus kirkii* in (A) lateral, (B) ventral and (C) dorsal view. bart, basal articulationof quadrate; dart, dorsal articulation of quadrate; fr, frontal; fv, vagus foramen; mmp, maxillary part of the maxillopalatine; na, nasal; nc, nasal capsule; ob, os basale; oc, orbital cartilage, pat, palatine tooth; pal, palatine; par, parietal; pcc, postchoanal commissure; pmp, palatine part of the maxillopalatine; pmx, premaxilla; ppt, pterygoid process of the quadrate; prf, prefrontal; prpp, postpalatinal process; q, quadrate; smx, septomaxilla; sq, squamosal; st, stapes; tdf, tentacular duct foramen; tf, tentacular foramen; vo, vomer. Scale bar equals 1 mm.

parasphenoid to form the os basale, similar to that of the adult except for some ossification deficits around the carotid foramen. A large, cartilaginous, bar-

shaped basal process articulates with the base of the pterygoid process of the quadrate. Another articulation between the os basale and the quadrate exists at the anterodorsal limit of the otic capsule, where a short, cartilaginous process articulates with the dorsomedial tip of the quadrate. Both articulations are rather loose in that the elements are somewhat separated but bound by connective tissue. A small, simple, rod-shaped cartilaginous stapes is found posterior to the quadrate. The quadrate has a cartilaginous articular facet for the stapes at its posterior edge, although both elements are not in contact but separated by a gap. All dermal bones are well developed. A squamosal is present, which covers the anterolateral aspect of the quadrate and slightly overlaps the prefrontal anteriorly. The squamosal has a loose articulation with the maxillary part of the maxillopalatine anteroventrally and leaves a broad temporal gap between it and the parietal and os basale medially, through which the *m. add. mand. longus* is visible. Nasal, frontal and parietal are similar in shape to the adult condition but not as well sutured medially, leaving the sphenethmoid partly exposed between the frontals and nasal. The septomaxilla and especially the prefrontal have become greatly expanded and are similar to those of the adult, except for the relatively wide sutures between the elements. The premaxilla is similar to that of the foetus, but distinctly more crescent-shaped in ventral view. Its dental lamina in particular is broader than in the adult, and the element as a whole is proportionately larger. The maxilla is fused with the palatine to form the maxillopalatine. It has a complex structure and consists of a broad, laterally expanded maxillary shelf that supports the maxillary and extraoral teeth seen at the lateral extremity of the paraoral process (note that most of the tooth-crowns have detached from their sockets during clearing and staining and subsequent preparation, and are omitted in Fig. 3). In lateral view, the maxillary part of the maxillopalatine has an almost wing-like shape, greatly increasing the depth of the anterior half of the skull. The palatine is broadly fused with the maxilla at its anterior end but both elements are still separated by a narrow gap further posteriorly. Part of the chondrocranium, on which the palatine rests, seems to have been incorporated into the palatine and especially the medial-most, posterior

part shows some ossifying cartilage and seems to represent the caudal process incorporated into the palatine. The anterior process of the vomer has expanded and a short, broad palatine shelf is present posterior to the vomerine tooth row, giving it a shape similar to that of the adult. Because of the expanded, posteroventrally directed dental shelves of the premaxilla and the maxilla part of the maxillopalatine, the premaxillary-maxillary arcade is in effect positioned much further ventral than the vomero-palatine arcade. This is in contrast to the adult, where both arcades are approximately at the same level.

The musculature of the juvenile is similar to that of the foetus. The *mm. add. mand. longus et articularis* are covered by the squamosal and *m. depressor mandibulae* and are barely visible in lateral view. The only more pronounced ontogenetic change in musculature is in the *m. interhyoideus posterior*, which has much expanded dorsally following the loss of the external gills. In all respects, the musculature of the juvenile is similar to the adult condition, except that the *m. intermandibularis* is proportionately larger in adults in association with the more elongated lower jaw. Additionally, the fibres of the *m. pterygoideus* are more steeply inclined in association with the extended and more dorsally bent retroarticular process in the adult.

Discussion

The skull morphology of adult *Scolecomorphus* has been repeatedly investigated in several species and is remarkably similar (Peter, 1895; Brand, 1956; Taylor 1969b; Nussbaum, 1985). It is apparent that both the foetal and juvenile stages of *Scolecomorphus kirkii* investigated here have a head morphology that differs remarkably from that of conspecific adults. The chondrocranium, and especially the nasal capsules, of the foetus is unexpectedly well developed and more robust than in embryos of other species investigated so far (e.g. Peter, 1898; Wake et al., 1985; Müller, 2006), where most of the elements are rather slender bars or thin plates that give the impression of a less robust structure than in *S. kirkii*. It is furthermore in stark contrast to the endocranium of adult *Scolecomorphus spp.*,

which have the most reduced nasal capsules among adult caecilians (Brand, 1956; Wake, 2003).

The mandibular arch musculature in *Scolecomorpus* is relatively simple, compared to other caecilians (e.g. Wilkinson and Nussbaum, 1997; Kleinteich and Haas, 2007). Only two primary jaw adductors are present, the *mm. adductores mandibulae longus et articuluaris*, while the *mm. adductores mandibulae externus et internus* are absent. A *m. levator quadrati* is also absent. Previously unreported in any *Scolecomorphus* is the presence of two *m. pterygoideus*-like muscles. In other caecilians, the single *m. pterygoideus* originates form either the pterygoid or the pterygoid process of the quadrate and inserts on the medial side of the retroarticular process of the lower jaw (Wilkinson and Nussbaum, 1997; Kleinteich and Haas, 2007). In *S. kirkii*, the smaller, anterior muscle originates from the posteriorly directed process, and later the maxillopalatine, via a strong fascia, while the larger, posterior one originates from the lateroventral neurocranium, just underneath and behind the basipterygoid process. Both pterygoideus-like muscles attach on the medial side of the retroarticular process of the lower jaw. A pterygoid is absent in *Scolecomorphus* and the pterygoid process of the quadrate is quite small and dorsally displaced. Based on topological relationships, it seems most likely that both muscles are derived from the single ancestral *m. pterygoideus* of other caecilians, which split and shifted its origin.

Function

The extent to which the adult skull of caecilians is kinetic has been discussed extensively (for a summary of the earlier literature, see Wake and Hanken, 1982). De Villiers (1938) and Brand (1956) considered the squamosal to be tightly bound to the prefrontal and, in the absence of a quadrato-stapedial articulation, interpreted the skull of *Scolecomorphus* to be monimostylic and therefore akinetic. Based on various species, models of caecilian skull kinesis have recently been proposed, which all consider the cheek region, consisting of the quadrate, squamosal and, to a varying extent, the maxillopalatine, to form a movable unit

(Straub, 1985; Wilkinson and Nussbaum, 1997). Wilkinson and Nussbaum (1997) discussed skull kinesis in *Atretochoana eiselti*, a large, lungless typhlonectid caecilian characterized by a uniquely derived morphology that includes a large, laterally projecting basipterygoid process and an absence of a quadrato-stapedial articulation, and concluded that these features greatly increase the mobility of the cheek region. It seems that a similarly increased mobility of the quadrate and squamosal is also realized in *Scolecomorphus*. Jones et al. (2006) suggested that increased skull kineticism in *Scolecomorphus* helps consuming large earthworms.

Specialized morphological structures have been discovered in foetuses of several viviparous caecilian species studied to date. Foetuses of almost all studied viviparous taxa have a specialized dentition thought to be used to scrape the oviduct lining (Parker and Dunn, 1964; Wake 2003). Similar teeth occur in *Scolecomorphus vittatus* (Loader et al., 2003) and *S. kirkii*. Because foetuses and juveniles show a dramatically different morphology of the premaxillary-maxillary arcade and associated structures compared to adults, it is tempting to speculate that these represent an adaptation to viviparity and are therefore likely connected to intraoviductal feeding in *Scolecomorphus*. Several lines of evidence however, suggest that the special morphology of foetal and juvenile *Scolecomorphus* is more likely to be related to post parturition feeding than to intraoviductal feeding. The lining of the oviduct does not seem to be hypertrophied as in other viviparous caecilians that exhibit intraoviductal feeding (e.g. Wake and Dickie, 1998). Specialized, so-called foetal teeth are now known to occur also in juveniles of direct developing caecilians and Kupfer et al. (2006; Chapter 5) recently suggested that "foetal" teeth may have first evolved in direct developing caecilians and were later co-opted for intraoviductal feeding in viviparous forms. The presence of specialized foetal and juvenile teeth is therefore not necessarily indicative of intraoviductal feeding in viviparous caecilians. In the foetus of *S. kirkii*, the tooth crowns of the premaxillary-maxillary and dentary arcade are furthermore still covered by the epidermis and thus non-functional, at least at this stage of development. In contrast, all teeth are erupted in the investigated

juveniles and show clear signs of wear. Juveniles of *S. vittatus* (Loader et al., 2003) and *S. kirkii* (this study) both had an amorphous, flaky, white substance in their hindguts, showing that juveniles of both species have apparently similar feeding habits that are distinct from the usual spectrum of primarily invertebrate prey found in adults (Jones et al., 2006). Kupfer et al. (2006) suggested that the form of post-parturition care and skin feeding seen in *Boulengerula taitanus* might have been a preadaptation to viviparity in other caecilians. In this regard, *Scolecomorphus* seems to be intermediate between direct-developing forms with "foetal teeth" and post hatching skin feeding like *B. taitanus* (Kupfer et al., 2006; Chapter 5), and viviparous forms with intraoviductal feeding and fully developed precocial young upon birth (Wake, 1977), in that it has a presumably shorter gestation period than viviparous forms with precocial young, and post-parturition feeding possibly associated with maternal care. The early ontogeny of *Scolecomorphus* seems to be important for our understanding of caecilian life-history evolution and should be particularly targeted in future studies on the evolution of viviparity in caecilians to test the evolutionary scenario proposed by Kupfer et al. (2006; Chapter 5).

Based on the then single known juvenile specimen of any *Scolecomorphus*, Loader et al (2003) discussed the possibilities that the peculiar morphology might be an adaptation to either or both the foetal or juvenile phase in the life history. As argued above, it is more likely that this unusual morphology is indeed an adaptation to post-parturition feeding. Recent fieldwork has resulted in the discovery of a highly specialized form of parental care in the direct developing *Boulengerula taitanus*, where juveniles feed on the modified skin of their mother and triple in size while under care (Kupfer et al., 2006; Chapter 5). Juveniles of *B. taitanus* are characterized by a specialized "foetal" dentition and show pronounced differences in skull development compared to other direct developing caecilians (H. Müller, pers. obs.; see Chapter 4). One of the main differences between foetal and juvenile *Scolecomorphus* and those of other caecilians – direct developing with or without post-parturition care and viviparous – however, is that the premaxillary-maxillary arcade forms a very broad arc that is oriented at a

large angle to the sagittal axis (Fig 5). This is because of an almost transverse orientation of the dental lamina of the premaxilla and the large, out turned maxillary arcade. Developmental changes in other caecilians are mainly due to a posterior extension of the maxillary arcade during ontogeny, but *Scolecomophus* has a very different orientation of the premaxillary-maxillary arcade during early life. It is, however, noteworthy that besides *S. kirkii*, the largest angles are seen in foetuses and juveniles of species known or suspected to scrape-feed in their early ontogeny. There seems to be a gradual decrease in angle between the foetal and the juvenile stage in *S. kirkii*. However, a large gap separates the juvenile and adult morphologies and it is at present unclear whether the transition between them is a gradual one or more climactic, metamorphosis-like, although it seems that the latter is more likely. The gap in total length between foetuses and juveniles are similar to that between the largest juvenile showing the particular morphology and the smallest adult-like specimen, the difference in orientation of the premaxillary-maxillary arcade between foetuses and juveniles is relatively small, whereas that between juvenile and adult morphology is much larger. It

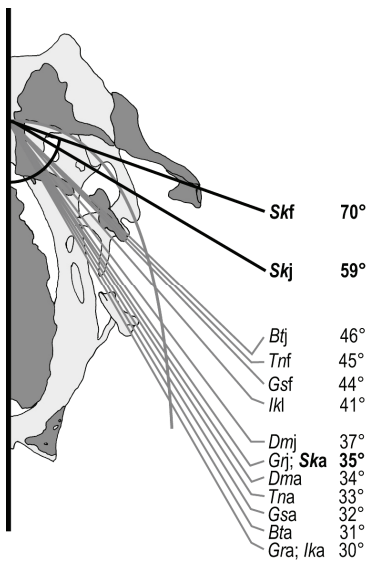

Fig. 5. Orientation of the premaxillary-maxillary arcade in various caecilian species plotted onto an outline drawing of the investigated foetus of *Scolecomorphus kirkii*, in ventral view. Lines indicate the angle of the premaxillary-maxillary arcade in various species and life-history stages, with the grey parabola representing the typical orientation of the arcade found in other caecilian species. Angles were measured from the medial end of the dental lamina of the premaxilla to the posterior, functional end of the dentary lamina of the maxilla or maxillary part of the maxillopalatine, usually indicated by the last tooth. Angles for *Dermophis mexicanus* measured from Lessa and Wake (1992), others from material in the collection of the BMNH. *Btj*, *Boulengerula taitanus* juvenile; *Bta*, *B. taitanus* adult; *Dmj*, *Dermophis mexicanus* juvenile; *Dma*, *D. mexicanus* adult; *Grj*, *Gegeneophis ramaswamii* juvenile; *Gra*, *G. ramaswamii* adult; *Gsf*, *Geotrypetes seraphini* foetus; *Gsa*, *G. seraphini* adult; *Skf*, *Scolecomorphus kirkii* foetus; *Skj*, *S. kirkii* juvenile; *Ska*, *S. kirkii* adult; *Tnf*, *Typhlonectes natans* foetus, *Tna*, *T. natans* adult.

appears therefore as if some accelerated transformation from the juvenile to the adult-like morphology occurs between 110 mm and 150 mm total length in *S. kirkii*.

This study underlines the distinctiveness of scolecomorphid caecilians, and *Scolecomorphus* in particular, which seem to be of special importance to our understanding of life-history evolution in caecilians. A better understanding of *Scolecomorphus* life-history is further important to test the scenario proposed by Kupfer et al. (2006; Chapter 5) for the evolution of viviparity in caecilians. Clearly, more observations especially of live animals are needed for further functional interpretations of the unusual juvenile morphology. Our observations also contribute to our understanding of the diversity of caecilian amphibians and, also in light of recent discoveries (Kupfer et al. 2006; Chapter 5), should encourage further study of caecilian developmental biology and life-history.

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Appendix
List of specimens examined. Museum collection acronyms: AMNH – American Museum of Natural History, New York; BMNH – The Natural History Museum, London

Taxon	Number	Life history stage	Size (in mm)	Preparation
Scolecomorphus kirkii	ex. BMNH2005.890	foetus	41	serial sections, 3D reconstruction, angles
	ex. BMNH2005.890	foetus	43	dissection, cleared and stained
	ex. BMNH2005.890	foetus	43	SEM
	AMNH A156899	juvenile	93	dissection, cleared and stained
	AMNH A156897	juvenile	104	–
	AMNH A156898	juvenile	106	–
	BMNH2005.895	subadult	159	–
	BMNH2005.894	subadult	209	–
	BMNH2005.891	adult	295	serial sections
	BMNH2005.893	adult	350	–
	BMNH2005.890	adult	402	–

CHAPTER 7 – SYNOPSIS

The caecilian skull

The skulls of caecilian amphibians are generally heavily ossified, probably as an adaptation to a burrowing life-style practiced by most members of the group (e.g. Peters, 1880; Marcus et al., 1933; Taylor, 1969a; Duellman and Trueb, 1986; Nussbaum and Wilkinson, 1989). In this respect, caecilian skulls differ from those of frogs and salamanders, which are generally less robust and usually have a very open cheek region (Duellman and Trueb, 1986; Trueb, 1993). Caecilians are further characterized by the fusion of individual bones into the larger compound bones typical of the adult caecilian skull (Peter, 1898), which complicates a direct comparison of caecilian adult skulls with those of frogs and salamanders and their putative Paleozoic ancestors. In cases where the adult morphology is highly derived, ontogenetic information is often useful in providing additional support for or against a certain hypothesis. In caecilians, study of the development of the skull is necessary for a better understanding of the adult morphology. Most previous studies of caecilian skull development were limited by the amount of material available (e.g. Marcus et al., 1935; Ramaswamii, 1948) or focused more on specific anatomical regions of the skull (e.g. Peters, 1898; Winslow, 1898; Jurgens, 1971; Wake et al., 1985). Wake and Hanken (1982) provided the first detailed description of the development of the skull in a caecilian, the viviparous caeciliid *Dermophis mexicanus*, and noted that many of the previously reported skull bones, such as quadratojugal or postparietal, do not occur. Wake and Hanken (1982) considered that part of the discrepancy between their and earlier studies, and especially that by Marcus et al. (1935), could be related to different life-histories in the investigated species, which is known to affect skull development in frogs and salamanders (e.g. Hanken et al., 1992; Wake and Hanken, 1996). Müller et al. (2005) studied the development of the skull in *Gegeneophis ramaswamii*, a direct developing species closely related to the species investigated by Marcus et al. (1935), and also found no indications of many of the previously reported multiple skull

ossifications, which raised considerable doubts about the validity of these studies. Müller et al. (2005) also demonstrated that the ossification sequence of the direct developing *G. ramaswamii* is considerably different from that of the viviparous *D. mexicanus* (Wake and Hanken, 1982).

In **Chapter 2** of this thesis, I reinvestigated skull development in *Hypogeophis rostratus*, which, together with the closely related *Grandisonia alternans*, had formed the subject of the ontogenetic studies by H. Marcus and his students (e.g. Eifertinger, 1933; Marcus et al. 1933, 1935). Although later studies pointed out inconsistencies (Brand, 1956) and, more recently, incongruence with other taxa (Wake and Hanken, 1982; Müller et al., 2005), the data reported by Marcus and students has been hugely influential in subsequent studies of caecilian skull morphology and amphibian evolution. The results of my investigation are largely incompatible with those of Marcus and students, and I find no evidence for several of the previously reported multiple ossifications, including supra-, infra- and basioccipital, epiotic, pleurosphenoid, preethmoid, posterior vomer, prepalatine, quadratojugal, postparietal, second coronoid, supraangular and complementare. It appears that most of Marcus et al.'s reports of non-existent ossifications are based on false phylogenetic preconception, misinterpretation of the observed morphology and technical error. The ossification sequence of *H. rostratus* is very similar to that of *G. ramaswamii*, although it is at present unclear whether this is related to their direct mode of development or close phylogenetic relationship.

Caecilians exhibit all the main reproductive modes known among frogs and salamanders – oviparity with a free-living larva, oviparity with direct development and viviparity (Wilkinson and Nussbaum, 1998). **Chapter 3** investigates the morphology the skull and associated musculature in larvae and adults of representatives of all lineages that are known to have free-living larvae, which presumably represents the ancestral state in caecilians. Despite several obvious differences in detail, larval caecilians share a very similar general morphology that is different from that of adult caecilians. All caecilian larvae are characterized by a gymnokrotaphic skull with a completely open temporal region

and a different shaped squamosal to that of the adults. In some taxa (rhinatrematids, ichthyophiids and *Praslinia cooperi*) the squamosal of larvae anchors the quadrate to the skull and has a similar form to that seen in most larval and adult salamanders and frogs (Trueb, 1993; Rose, 2003). Larval caecilians further show a palatal metamorphosis that includes a posterior elongation of the maxilla. As with cranial morphology, larval cranial musculature and hyobranchial morphology is more similar among larvae of different species than between larvae and adults and musculature and the hyobranchial skeleton also undergo metamorphic changes similar in degree to those seen in the skull. This account provides the first descriptions of the skull and hyobranchial skeleton of larval *Uraeotyphlus* spp., larval and adult *Praslinia cooperi*, larval *Sylvacaecilia grandisonae* and larval *Grandisonia spp.*, as well as of the musculature of larval *Epicrionops spp.* and *Rhinatrema bivittatum*, larval *Uraeotyphlus spp.*, larval and adult *P. cooperi*, larval *S. grandisonae* and larval *Grandisonia spp.*

The postembryonic development of the skull in two distantly related direct developing caecilian species with different life-history strategies is investigated in **Chapter 4**. We focussed on the postembryonic development of the skull in *Boulengerula taitanus*, a caeciliid with an extended period of post-hatching parental care, and the caeciliid *Gegeneophis ramaswamii*, which lacks post-hatching parental care. Postembryonic skull development in these two taxa was compared with that of *Ichthyophis* cf. *kohtaoensis*, a species with a free-living aquatic larva, the presumed ancestral condition in caecilians. Compared with *G. ramaswamii*, hatchling *B. taitanus* have a far less developed skull and are unlikely to be able to burrow. Skull development, especially the closure of the cheek region continues during the early postembryonic phase. The general trajectory of skull development in *B. taitanus* is nonetheless similar to that of *G. ramaswamii*, indicating a heterochronic shift in hatching time in the former. Skull development in both species is further characterized by the absence of larval-specific traits seen in larvae of *I.* cf. *kohtaoensis*, which shows that direct development in caecilians is also characterised by ontogenetic repatterning.

Chapter 5 is a closer examination of the unusual form of parental care present in *Boulengerula taitanus*. Although the initial growth and development of most multicellular animals depends on the provision of yolk, there are many varied contrivances by which animals provide additional or alternative investment in their offspring (Clutton-Brock, 1991). Providing offspring with additional nutrition should be favoured by natural selection when the consequent increased fitness of the young offsets any corresponding reduction in fecundity (Smith and Fretwell, 1974). Alternative forms of nutrition may allow parents to delay and potentially redirect their investment. *Boulengerula taitanus* exhibits a remarkable form of parental care and mechanism of parent-offspring nutrient transfer. In this direct developing, oviparous caecilian (Nussbaum and Hinkel, 1994), the skin is transformed in brooding females to provide a rich supply of nutrients for the developing offspring. Young animals are equipped with a specialised dentition, which they use to peel and eat the outer layer of their mother's modified skin. This new form of parental care provides a plausible intermediate stage in the evolution of viviparity in caecilians. At independence, offspring of viviparous and oviparous dermatotrophic caecilians are relatively large despite being provided with relatively little yolk. The specialised dentition of skin-feeding (dermatophagous) caecilians may constitute a preadaptation to the foetal feeding on the oviduct lining of viviparous caecilians.

Chapter 6 describes the external and musculoskeletal morphology of the head in an ontogenetic series of the scolecomorphid caecilian *Scolecomorphus kirkii*. The rostral region of foetuses and juveniles in this viviparous species is expanded into large, posterolaterally pointing paraoral processes that are formed by the maxilla. Extraoral teeth that show signs of wear are present on the underside of the rostrum in front of the mouth and laterally on the paraoral processes. In foetuses the extraoral teeth are covered by epidermal tissue, which suggests that the peculiar morphology is indicative of a specialized post parturition feeding stage in *Scolecomorphus* (see also Loader et al., 2003). The endoskeletal part of the foetal skull is largely cartilaginous, while all of the dermal bones, with the exception of the squamosal, are well developed. The

foetal chondrocranium is extensively developed and shows a peculiar posteriorly directed process of the lamina orbitonasalis posterolaterally, which is joined by a transverse bar joining the pila preoptica posterior to the choana, and a posteriorly directed lateral process (postpalatine process) that extends parallel to the trabecular cartilage beyond the level of the posterior end of the pila antotica. A similar chondrocranial morphology is unknown from any other caecilian taxon. Only two primary jaw adductor muscles are present, together with two pterygoideus-like muscles that insert onto the lower jaw. The palatoquadrate, respectively quadrate of foetuses and juveniles shows a high degree of mobility. Compared with the limited data available on skull development in other viviparous species Wake and Hanken, 1982; Reiss and O'Reilly, 1999), results suggest profound diversity in early skull development and highlight the need for more comparative data on viviparous caecilians.

Phylogenetic position of caecilians

Much has been written about the phylogentic relationships between caecilians and other amphibians, Recent or fossil, and much will undoubtedly be written in the future. Of the various hypotheses put forward regarding caecilian relationships (e.g. Kingsley, 1902; Marcus et al., 1935; Nussbaum, 1983; Løvtrup, 1985; Carroll and Currie, 1975; Laurin and Reisz, 1997; Anderson, 2001; McGowan, 2002; Carroll et al., 2004; Schoch and Milner, 2004) only three are currently being considered seriously. Two of these recover Lissamphibia as a monophyletic group that has its origin from either among the Temnospondyli (e.g. Parsons and Williams, 1963; Milner, 1988; Bolt, 1991; Trueb and Cloutier, 1991; Ruta et al., 2003; Schoch and Milner, 2004; Ruta and Coates, in press) or the Lepospondyli (Laurin and Reisz, 1997; Laurin, 1998). Most authors favour a temnospondyl origin of Lissamphibia, although support for the lepospondyl hypothesis is not significantly worse (Ruta and Coates, in press). The third hypothesis, which postulates a separate origin of frogs from temnospondyls and caecilians from lepospondyls, with salamanders variously derived from either group, has repeatedly been proposed (Carroll and Currie, 1975; Carroll, 2000; Schoch and

Carroll, 2003; Carroll et al., 1999, 2004), but received no support in any of the recent quantitative phylogenetic analyses (see above).

In this regard, it is worthwhile stressing again the phylogenetic importance of metamorphosis in all Recent amphibians (Schoch and Milner, 2004). Caecilians share with frogs and salamanders a similar suite of metamorphic patterns, ranging from changes in the soft anatomy, such as the epidermal structure (Duellman and Trueb, 1986) or cranial musculature (Bauer, 1997; Haas, 2001), to metamorphic changes in the skeleton (Duellman and Trueb, 1986). Cranial changes include the conspicuous posterior elongation of the maxilla upon metamorphosis and changes in the palatal region, including a widening of the interpterygoid vacuities (Reiss, 1996, 2002; Schoch and Milner, 2004). Frogs and salamanders are further characterized by a more pronounced condensation of ossification events into the metamorphic period (Roček and van Dijk, 2006; Schoch, 2002a, 2002b). Caecilians differ in that the larval skull has more or less the adult set of bones already upon hatching, but they undergo extensive fusion and remodelling of certain bones, such as maxilla and squamosal, upon metamorphosis (see **Chapter 3**). However, the circumorbital of ichthyophiids and uraeotyphlids only forms during metamorphosis, and the formation of the maxillopalatine in rhinatrematids, which might also incorporate a postfrontal element, is unknown.

Looking at the fossil record, several temnospondyl groups exhibit ontogenetic changes that are reminiscent of the metamorphic changes seen in Recent amphibians (Schoch 2002a) albeit far less pronounced. The enlargement of the interpterygoid vacuities, for instance, also seems to characterise certain temnospondyls (Reiss, 2002). However, the foreshortened maxilla of larval Recent amphibians and its posterior elongation during metamorphosis is not found in any Palaeozoic amphibian group (Schoch 2002a) and seems to be a possible lissamphibian synapomorphy (Reiss, 2002). The temnospondyl branchiosaurid *Apateon* furthermore exhibits a cranial ossification sequence that is very similar to that of salamanders (Schoch 2002b; Schoch and Carroll, 2003), although a frustrating lack of data on other relevant groups prohibits further

conclusions to be drawn. Ontogenetic studies of lepospondyls are scant because of a general absence of suitable material, although the few available data seem indicate a complete absence of metamorphosis-like changes in cranial ontogeny (Anderson, 2003). It is also of importance to note that metamorphic changes reminiscent of lissamphibians are only found within the Dissorophoidea and not in all temnospondyls (Schoch, 2002a). Dissorophoidea comprises several taxa, such as branchiosaurids and doleserpetontids, regarded as closely related to lissamphibians, or at least some subtaxa of lissamphibians (e.g. Parsons and Williams, 1963; Milner, 1988, Trueb and Clouthier, 1991; Schoch and Carroll, 2003; Schoch and Milner, 2004). Ontogenetic studies indicate a progressive condensation of metamorphic events in dissorophoids as compared to other temnospondyls (Schoch 2004; Witzmann and Pfretzschner, 2003; Witzmann, 2005) and it seems likely that metamorphosis evolved within a taxon comprising dissorophoids and lissamphibians. The greater condensation of metamorphic events in lissamphibians as compared to dissorophoids seems to have occurred partly within the lissamphibian stem-line and partly within crown group lissamphibians (Roček and van Dijk, 2006).

Carroll (2000) and Anderson (2001) considered *Eocaecilia* and, by implication, caecilians to be nested within Microsauria. The presence of metamorphosis in caecilians, however, is a strong indication of a close relationship with both frogs and salamanders, as well as with certain temnospondyls, rather than lepospondyls. Schoch and Milner (2004) have further pointed out various dissorophoid features of *Eocaecilia* and regarded the similarities of *Eocaecilia* and the microsaur *Rhynchonkos* to be associated with a similar, burrowing life-style and not necessarily indicative of common descent. All this would speak for a temnospondyl origin of caecilians, rather than for lepospondyl affinities, and discount both hypotheses of a diphyly or lepospondyl origin of lissamphibians (e.g. Carroll and Currie, 1975; Laurin and Reisz, 1997; Laurin, 1998; Carroll, 2000; Carroll et al., 1999, 2004).

Ontogentic data further clearly indicate that caecilians have a reduced set of skull bones (Wake and Hanken, 1982; Müller et al., 2005; Müller, 2006; see

Chapter 2) similar to frogs and salamanders, and that the heavily ossified caecilian skull likely evolved secondarily as an adaptation to a burrowing life-style from a gymnokrotaphic or zygokrotaphic ancestor (see **Chapter 3**). This raises questions regarding the caecilian affinities of the putative stem-line caecilian *Eocaecilia micropodia*. An open temporal region is present in the earliest known stem-group representatives of frogs and salamanders (and their descendants), and we have argued that a similar condition is also characteristic of the last common ancestor of living caecilians (**Chapter 3**). The Albanerpetontidae, a group of small, salamander-like forms that has been variously placed as stem-group salamanders, stem-Batrachia, stem-Lissamphibia, or sistergroup to Gymnophiona (Milner, 1988; Gardner, 2001; McGowan, 2002; Ruta et al., 2003; Ruta and Coates, in press), also possessed an open temporal region. Accepting *Eocaecilia* as a stem-line caecilian implies convergent evolution of a zygokrotaphic skull in caecilians and other lissamphibians, as well as convergent loss of various skull bones (Schoch and Milner, 2004; but see also Ruta et al., 2003). Considering the characters that link *Eocaecilia* and modern caecilians, it is noteworthy that several of these are linked to a burrowing life-style and therefore possibly more prone to convergence as has been argued for similarities between *Rhynchonkos* and *Eocaecilia* (Schoch and Milner, 2004). This raises the possibility of an alternative placement of *Eocaecilia* within dissorophoids, but removed from the immediate ancestry of caecilians. Further investigation of the relationships of caecilians and *Eocaecilia* seems warranted and should receive particular attention in future studies of temnospondyl and lissamphibian phylogeny.

Future work

Recent work including this thesis (e.g. Wilkinson, 1996; Wilkinson and Nussbaum, 1996; O'Reilly et al., 1997; Loader et al., 2003; Kupfer et al., 2006; Chapters 2, 3, 4, 5, 6) has demonstrated in an impressive way a previously unsuspected diversity of caecilian amphibian biology, ranging from morphology to life-history evolution. In the general amphibian literature, however, caecilians

have traditionally been considered to be a fairly homogenous group with little differentiation among its members. Somewhat paradoxically, this view has apparently been held not because, but despite of previous research or the lack thereof. The prevailing notion in the scientific literature seems to be that caecilians are rare, difficult to collect and somehow remarkably similar to each other, although a similar statement could be similarly applied to such intensively studied groups as whales and dolphins. The research presented in this thesis has – yet again – shown a remarkable diversity of caecilians in developmental morphology and other aspects of their biology. However, in as much as recent work has improved our understanding of caecilian biology, there are still large gaps in our current knowledge, especially regarding the basic biology of numerous species and their phylogeny, which frustratingly hamper further progress at the moment. Active fieldwork seems to be the key to continued and fast progress in caecilian research. For instance, further fieldwork, combined with behavioural observations, seems almost guaranteed to provide decisive evidence on the function of the peculiar morphology of juvenile *Scolecomorphus* described in **Chapter 6** and by Loader et al. (2003). A similar assumption seems justified in view of the function of the peculiar foetal dentition in non-viviparous caecilians (**Chapter 5**) and its possible association with skin feeding in species other than *Boulengerula taitanus* (as predicted in Chapter 5). Further research should focus on skull development in additional taxa, and especially rhinatrematids because a better understanding of their development is likely to advance our understanding on the phylogenetic position of caecilians more than previously studied groups (see **Chapter 3**). More and detailed ontogenetic studies would also provide more data to test evolutionary trends within caecilians in a more detailed way than currently possible. A particular emphasis should be placed on direct developing and viviparous species to test for hypotheses of independent life-history evolution (**Chapter 3, 4, 5**). Caecilians would further make a promising target for the study of body elongation and limb reduction in tetrapods and seem well suited to complement genetic and morphological studies in other limbless and elongated groups such as snakes (Cohn and Tickle, 1999). Again, this is most likely

achieved by increased future field work efforts. Although recent years have seen an advance in or understanding of caecilian intrarelationships (Gower et al., 2002; Wilkinson et al., 2003; San Mauro et al., 2004; Frost et al., 2006; Roelants et al., 2007) and especially an emerging consensus on the relationships within the Caeciliidae, the largest and most diverse group, more effort is needed to establish the robust phylogenetic framework needed for the study of caecilian evolution. Increased fieldwork will generate more samples for these taxonomic and systematic studies. Taxa exhibiting interesting but understudied life histories, such as *Sylvacaecilia grandisonae* with free-living larvae or the direct-developing, miniaturized *Idiocranium russeli* (Wake, 1985) should be targeted in future fieldwork.

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Curriculum vitae

Hendrik Müller was born on 12. December 1975 in Berlin, Germany. He entered the 4. Polytechnic School Berlin Weissensee in 1982. In 1990 he transferred to the newly established 1. Grammar School Berlin Weissensee "Wieland Herzfelde", where he obtained his Abitur in 1995. In the same year, he enrolled in Biology at the Humboldt-University Berlin and obtained his Vordiplom in 1997. For his Diplom studies he chose zoology as a major and developmental biology and palaeobiology as minor subjects and graduated in 2002 as a Diplom-Biologe with a thesis on skull development in two caecilian species. The work on his diploma thesis was performed at the Institut für Systematische Zoology of the Museum für Naturkunde of the Humboldt-University. He subsequently received a PhD stipend from the Department of Zoology, The Natural History Museum, London, UK, and work on his PhD commenced on 01. January 2003 under the supervision of Drs. Mark Wilkinson and David Gower. Shortly afterwards he was admitted to the PhD program at Leiden University, The Netherlands as an external PhD student under the supervision of Dr. Michael Richardson.

Professional experience

1998-2000	Teaching Assistant for undergraduate course "Cytology" and Research Assistant in the Laboratory for Electron Microscopy and Histology, Department of Molecular Parasitology, Humboldt-University at Berlin
2000-2001	Research Assistant at the Hermann-von-Helmholtz-Centre of Cultural Technology, Humboldt-University at Berlin, concerned with preparing part of the Zoological Teaching Collection of the Institute of Biology for presentation in the "Theatrum naturae et artis" exhibition
2001-2002	Research Assistant at the Hermann-von-Helmholtz-Centre of Cultural Technology, concerned with assessing various collections of the Humboldt-University in an integrated database for multidisciplinary use
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Teaching

1998-2000	undergraduate course "Cytology", Humboldt-University at Berlin, Germany
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2003	Katharina-Heinroth Award of the Gesellschaft Naturforschender Freunde zu Berlin (gegr.1773) [Berlin Society of Researching Friends, founded 1773] for outstanding Diploma [MSc] Thesis
2003-2006	PhD studentship for the duration of three and a half years by the Department of Zoology, The Natural History Museum London
2004	NSF travel grant to attend the Amphibia Tree Workshop in Austin, Texas, 3.-4. December 2004
2005	Systematics Association student bursary to attend the Systematics Association Biennial Conference in Cardiff, Wales, 22.-26. August 2005
2006	European Union SYNTHESYS collection study grant to visit the collection of the Natural History Museum Berlin for 3 weeks
2006	Named collaborator on DAPTF grant “The endemic amphibians of the Nguru Mountains, Tanzania: an integrated study of an Eastern Arc Mountain hotspot” to Dr. Simon Loader to survey the amphibian fauna of the Nguru Mountains, Tanzania
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3. **Müller, H.** (2003) Skull development in *Hypogeophis rostratus* and *Gegeneophis ramaswamii* (Amphibia: Gymnophiona) and its bearing on caecilian intra- and interrelationships.
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Conference presentations and seminar talks:

"Major events in early vertebrate evolution: palaeontology, phylogeny and development." a joint Systematics Association/NHM meeting held at the NHM London, 8./9. April 1999. Poster presented: Bartsch, P. & H. Müller: On the ontogeny of the autostylic jaw suspension in the Dipnoi".

6th International Congress on Vertebrate Morphology. Jena, Germany, 21.-26. July 2001. Poster presented: Bartsch, P. & H. Müller: The ontogeny of the skull in the Recent dipnoan genera revisited. published in J. Morphol. 248: 204.

Annual Meeting of the German Zoological Society. Halle, Germany, 20.-24. May 2002. Talk given: On the ontogeny of the skull of *Gegeneophis ramaswamii* (Amphibia: Gymnophiona). published in Zoology (105), Suppl. V (95.1):36.

Annual Meeting of the German Zoological Society. Berlin, Germany, 09.-13. June 2003. Poster presented: New observations on the disparate early life history and morphology of *Geotrypetes seraphini* (Amphibia: Gymnophiona: Caeciliidae).

4th Biennial Meeting of the Systematics Association, Trinity College Dublin, Ireland, 18.- 22. August 2003. Talk given: Skull development in *Hypogeophis rostratus* and *Gegeneophis ramaswamii* (Amphibia: Gymnophiona) and its bearing on caecilian intra- and interrelationships.

5th World Congress of Herpetology, University of Stellenbosch, South Africa, 19.-24. June 2005. Invited speaker in the “Caecilian symposium in honour of Werner Himstedt”, talk given: Caecilian skull development revisited.

5th Biennial Meeting of the Systematics Association, Cardiff University and the National Museums and Galleries of Wales, Cardiff, UK, 22.- 26. August 2005. Talk given: Skull development in caecilians and its implications for the reconstruction of the ground pattern of the caecilian skull.

6th Argentinean Congress of Herpetology, Parana, Argentina, 21.-23. November 2005. Poster presented: Evolution of direct development in caecilian amphibians, a case study

Institut für Systematische Zoologie, Naturkundemuseum Berlin, Germany, 21. April 2006. Invited seminar speaker: Schädelentwicklung bei Blindwühlen (Amphibia: Gymnophiona): systematische und evolutionäre Aspekte.

Faculty of Mathematics and Natural Sciences, Leiden University, The Netherlands, 20. June 2006. Invited speaker for the “Discoveries” seminar series of the faculty: Skull development in caecilian amphibians: systematic and evolutionary implications.

First and Founding Meeting of the European Society for Evolutionary Developmental Biology, Prague, 16.-19. August 2006. Invited symposium speaker: Development in caecilian amphibians; and poster co-presentation:

Evolution of the genetic and developmental basis of limblessness in caecilian amphibians.

Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum, Friedrich-Schiller-Universität Jena, Germany, 16. November 2006. Invited seminar speaker: Schädelentwicklung bei Blindwühlen (Amphibia: Gymnophiona): systematische und evolutionäre Aspekte.

Palherp 2007 Meeting, Staatliches Museum für Naturkund Stuttgart, Germany, 12.-13. May 2007. Talk given: Schädelevolution bei Blindwühlen.

8th International Congress on Vertebrate Morphology. Paris, France, 16.-21. July 2007. Invited symposium speaker: Morphology of larval caecilians (Amphibians: Gymnophiona); and poster presentation: Development of the skull in the Chinese soft-shelled turtle *Pelodiscus sinensis* (Reptilia: Testudines: Trionychidae).